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A New Nomenclature for the Chaetotaxy of the Mosquito Pupa, Based on a Comparative Study of the Genera (Diptera: Culicidae)[1]

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Until rather recently the mosquito pupa has usually been neglected by taxonomic workers. However, as the number of known mosquito species has increased, the necessity for additional species identification characters has caused an increased amount of attention to be directed to the pupal stage, with the result that it has frequently been found to possess good differentiating characters.

Early studies of the mosquito pupa (reviewed by Ingram and Macfie, 1917) disclosed that its rather elaborate chaetotaxy supplied an important source of pupal taxonomic characters. From that time on various systems of nomenclature have been proposed for this chaetotaxy.

A morphological nomenclature, expressive to the greatest extent possible of homologies between species, is, of course, absolutely essential to the taxonomist. To be completely adequate such a nomenclature should also be indicative of homologies from segment to segment within the individual. Such an ideal nomenclature is best obtained by doing a broad preliminary comparative study of representative members of the group being considered. Unfortunately, the nomenclature of mosquito pupal chaetotaxy in common use today did not develop in this manner and, as a result, when previously unstudied genera are met with, the nomenclature is frequently most difficult to apply.

For at least two additional reasons all of the available systems of pupal hair designations are considered by us to be unsatisfactory. First, none of them include all of the known elements of the chaetotaxy; and second, the systems all employ mixed types of name designations (words, numerals, symbols, and letters), a condition which introduces unnecessary difficulties into the mechanical handling of the nomenclature.

The work reported on here was undertaken in an attempt to devise a pupal hair nomenclature based on a comparative study of all the genera of the subfamily Culicinae. All of the pupal hairs and hairless setal rings have been considered, and every effort was taken to make the proposed nomenclature as mechanically simple as possible.

HISTORICAL

Although morphological studies of the mosquito pupal stage were made as early as 1901 by Nuttall and Shipley, no systematic consideration was given to the

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chaetotaxy until some years later. Macfie (1920) made the first thorough examination of the setae occurring on the pupa, using *Aedes aegypti* (L.) for the study. In his basic and excellent work, he found every seta at present known to occur on that species, overlooking only the dorsal hairless setal ring on abdominal segments III to V. Unfortunately, the nomenclature devised by him used rather lengthy and quite unwieldly word names which have since fallen into disuse. However, some of the capital letters used to label his figures of the abdominal hairs have been carried along and still persist in the nomenclature most commonly used today (the A, B, C hairs for example). In a slightly later paper, Macfie and Ingram (1920) applied the same nomenclature to the pupa of a *Culex* species.

Senevet (1930) developed an extensive modification of Macfie's terminology for use with anopheline pupae. Christophers (1933) slightly modified Senevet's anopheline nomenclature and it is this modification which is in common use for anophelines at the present time.

Crawford (1938) pointed out that Senevet's treatment of the ventral abdominal setae of anophelines is inconsistent with the conditions actually observable, and therefore proposed a new nomenclature for these setae (using word names).

Baisas (1938), using species of *Culex* (*Culex*), *Aedeomyia*, and *Aedes* (*Stegomyia*), attempted to modify the Christophers' nomenclature so that it could be used with both anophelines and culicines.

Edwards (1941) was the first to prepare a really comprehensive series of culicine pupal descriptions. For a nomenclature of the chaetotaxy he accepted that of Baisas (1938), changing only the designation of the large dorsal plumose hair of the first abdominal segment from "dendritic tuft" to "float hair."

MATERIALS AND METHODS

The project reported on here was initiated by obtaining slide-mounted pupal skin specimens of as many of the known mosquito genera and subgenera as possible. Representatives of only two of the 30 recognized mosquito genera were unobtainable: *Heizmannia* Ludlow[3] and *Paraëdes* Edwards.[4] Of the known subgenera, slightly more than one-half were represented in the material examined.

Next, the abdominal segments of at least one species of each of the 28 represented genera were illustrated (the metanotum has been included in these drawings only because it is normally associated with the abdomen in the method of dissection used). In two cases (*Uranotaenia* and *Mansonia*), where significant subgeneric differences were found within a genus, drawings of each of the involved subgenera were prepared. The arrangement of the cephalothoracic hairs was found to be so constant throughout the subfamily that only five illustrations of the cephalothorax (exclusive of the metanotum) were made.

By a combined use of the specimens and drawings a comparative study of the chaetotaxy of the entire subfamily was then made. The purposes of this study were: 1) to determine if each seta of one body segment of the pupa possessed determinable homologues on each of the other segments of the individual, and 2) to determine if each seta of each body segment of the pupa possessed determinable homologues on the equivalent segments in all the other genera of the subfamily. Positive findings for both of these points should then theoretically allow the application of a relatively stable nomenclature to the pupal setae.

[3] Mr. P. F. Mattingly has kindly consented to prepare and publish a drawing of the pupal skin of a species of this genus from type material that exists in the British Museum (Natural History). This is to appear in the Proc. Roy. Ent. Soc. London (A) in the near future.

[4] In the original description of *Paraëdes*, Edwards (1934) states that the early stages are unknown.

The criteria used for the determination of homologies between the elements of the pupal chaetotaxy were: 1) relative position, and 2) degree of development and general appearance. It must necessarily follow of course, that homologies based only on these two points will be subject to error, but until additional or better criteria are discovered these must largely do.

An attempt was made to furnish other criteria by making a rather extensive examination of a number of representative pupae of the subfamily Chaoborinae, which is regarded by some authors to be more primitive in development than the Culicinae, but nothing of help was found. An effort made to locate primitive or generalized setal arrangements by following the phyllogenetic generic arrangement proposed by Edwards (1932) was also unsuccessful. Rather, the result of such studies is to show the pupa to be a highly plastic life-cycle stage that is more apt to reflect the environment inherited by it from the larval stage than it does its species phyllogeny.

The pupal stage is best studied from the cast skin which is firm enough to mount in balsam and still retain its complete shape. However, for proper study, the skin should be dissected before the cover slip is added. This is perhaps best done by inserting a needle between the junction of the metanotum and the cephalothorax proper and separating these two structures in such a way as to leave the metanotum attached to the abdomen. The remainer of the cephalothorax now opens along the dorsal longitudinal midline and can be easily laid out flat with the outer surface up.

Care must be used in studying pupal chaetotaxy to avoid being confused by anomalous and evanescent structures. Such confusion can usually be guarded against by examining both the right and left sides of a specimen. A noticeable amount of natural variation in the position of hairs also occurs, the extent of which is rather difficult to determine because the abdominal skin seldom lies perfectly flat in the mounting medium.

RESULTS AND DISCUSSION

Since no means of establishing homologies between the cephalothoracic and abdominal hairs were discovered, they are discussed separately.

Cephalothoracic Setae. The arrangement of the cephalothoracic setae was found to be remarkably constant throughout the subfamily but the degree and type of development, however was variable.

Although no satisfactory homologies could be established between the cephalothoracic and abdominal setae, it is true that in several genera the appearance and arrangement of the three metanotal setae are such as to suggest a definite relationship between them and hairs 2, 3, and 4 on abdominal segment I. However, nearly as definite a relationship is also apparent in other genera with hairs 7, 8, and 10 of segment I. Since neither relationship could definitely be settled upon, the metanotal setae have been treated along with the other cephalothoracic setae as unrelated to those of the abdomen.

No apparent segmental relationships were found within the cephalothoracic chaetotaxy itself.

As pointed out by Macfie (1920), there are 12 pairs of cephalothoracic setae, one member of each pair being on either side of the midline. These setae occur in 4 natural groups. An examination of pupae nearly ready for adult emergence shows that the three anteroventral setae (the post-ocular setae of Macfie, 1920) are borne on the cephalothoracic sheath over the adult head, that the group of four setae dorsal to the antennal sheath (antero-thoracic setae of Macfie, 1920) and the group of two setae immediately posterior to the trumpet (dorsal and supra-alar setae respectively of Macfie, 1920) are all associated with the meso-

thorax, and that the three metanotal setae (postero-thoracic setae of Macfie, 1920) are over the postnotum.

The setae of the cephalothorax have been designated by us with arabic numerals (1–12), beginning with the most anterior group (the head group) and naming laterally and posteriorly from the dorsal midline in each succeeding group (see Figs. 31–35).

ABDOMINAL SETAE. In general, it was found possible on the basis of similarities in position and development[5] to establish homologies for the setae of abdominal segments I to VII, both between the hairs of each segment of the individual and between the hairs of equivalent segments of the different genera. However, in structure and in number of setae present, segments VIII to X[6] are so extensively modified in relation to each other and to the other abdominal segments that no adequate means of determining the homologies of their greatly reduced chaetotaxy were found.

Segment I is also modified in structure and in number of setae present, but no particular difficulty is experienced in determining the relationships of the remaining setae with those of the succeeding segments. Segment II is modified in that it normally possesses a reduced number of ventral setae, but as with I the relationships of the setae present are definitely apparent.

Excluding occasional evanescent hairs and hairless setal rings, the unmodified abdominal segments (III to VII) each possess a maximum of 13 pairs of dorsal, lateral, and ventral hairs, one member of each pair being on either side of the midline. In addition a hairless setal ring is present in most genera on segments III to V. The modified segments range from being hairless to possessing 12 pairs of setae.

To facilitate the task of establishing relationships between the hairs of abdominal segments I to VII, and to make it possible for other workers to trace these derived relationships, an attempt was made to find a key segment from which to proceed in recognizing affinities between the hairs of each segment. An examination of the species used for this study indicated that, of the segments possessing the maximum complement of setae, segment VI seemed to exhibit less variation in hair development and position throughout the subfamily than did any of the others. And indeed, it was found that by beginning with the sixth segment and proceeding in either direction that hair relationships could usually be more readily recognized and justified than by beginning with any of the other segments. Accordingly, this method was the one finally adopted for working out the relationships of the dorsal setae of segments I to VII (the relationships of the true ventral abdominal setae are rather uniformly apparent throughout the subfamily).

By use of the method for determining hair relationships described above, a nomenclature of arabic numerals was then applied. In applying the nomenclature, the dorsal hairless setal ring usually occurring on segments III to V was named 0, and the hairs were numbered 1 through 13, beginning at the dorsal midline and extending laterally and ventrally to the ventral midline. Due to the extensive modification of segments VIII to X, all of the hairs of those segments except 1 and 13 of VIII were arbitrarily assigned numbers. As mentioned previously, the modification of segments I and II is not sufficient to obscure the relationships

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[5] It should be stressed here that determinations of relationships based on the development and appearance of hairs are better done from specimens than from drawings, since the characters are frequently so subtle as to be difficult to illustrate perfectly.

[6] Following Edwards (1941), the anal flap and the paddles are regarded as representing abdominal segment IX, and the genital pouch as representing segment X.

of the hairs on those segments. It should be borne in mind that no homologies are implied between cephalothoracic and abdominal hairs which bear the same numbers.

For the purposes of discussing the abdominal setae and for clarifying their derived relationships, the modified segments (I, II, VIII, IX, and X) are treated separately from the unmodified segments (III–VII).

Unmodified Segments. On the unmodified segments (III–VII) all of the hairs except 1 and 13 fall into rather well-defined groups, and although almost every variation can and does occur in the development of the various hairs and hair groups, some definitive statements can be made.

Hairs 1 and 13 are on the anterior half of the segments and all the other hairs are in general on the posterior half of the segments.

The dorsal hairless setal ring (designated 0, but not labeled on the plates) of segments III–V is associated either with hair 4, 5, or 6, or with any combination of these. It is entirely missing in the species of *Trichoprosopon* and *Sabethes* studied, and absent from segment III of *Wyeomyia.*

Hair 1 is a remarkably uniform dorsal microseta (in occasional species showing a greater development, however), situated submedially near the anterior margin and well isolated from the other dorsal hairs.

Hairs 2, 3, and 4, which form a rather definite dorsal group in most genera, occupy the most medial position. Hair 2 (the C hair of authors) is usually well developed, and is located on or near the posterior margin of the segment. Excluding hair 1, either 2 or 3 occupies the most median location on the dorsum of each segment; the only consistent exception to this known to us occurs in the genus *Anopheles* where hair 4 on segment VI lies internal to either 2 or 3. In all of the species illustrated here, hair 2 is longer than hair 3 except in *Zeugnomyia* and *Eretmapodites.* Another distinct character of hair 2 on the unmodified segments is that it is rather constant in development and position on each segment of an individual (notable exceptions however are *Megarhinus* and *Trichoprosopon*). What is believed to be hair 2 is missing entirely from segments III–VII of *Mansonia* (*Coquillettidia*). The most striking character of hair 3 (hair C′ of authors) is that it is usually small, single, and slightly to strongly spinose in character (branched and truly hair-like only in *Bironella, Anopheles,* and some *Tripteroides*). In addition, hair 3 is constant in appearance, and very nearly constant in position, on each segment. Hair 4 (hair 4 of authors) is the most difficult of the abdominal hairs to define because of its extremely variable nature, both in development and position. Actually, it is only transiently a group associate with hairs 2 and 3. For example, on segment IV it is associated with the 5–6 group, and frequently also on other segments. In some cases the setal pattern of segment III shows a definite similarity with that of the modified segments I and II, and in these cases, one finds hair 4 retaining the prominent development and the same relative position as on segment II (see *Eretmapodites*). In general, the selection of hair 4 on the unmodified segments is made much easier by first selecting hairs 2, 3, 5, and 6. What is believed to be hair 4 is absent from VII of *Ficalbia* and *Mansonia* (*Mansonioides*).

Hairs 5 (B hair of authors) and 6 (2 hair of authors) form a rather definite group (frequently joined by 4 as pointed out above), which occupies a dorsal postero-sublateral position on the segment, external to the 2, 3, 4 group. Except for variations which may occur on segment III, hair 5 is posterior to 6; and, except on segment VII (and rarely VI) in a number of genera, it is more developed than 6. Frequently, hair 5 on segment III is markedly less developed than on the following three segments. Because of a well-developed 4 hair in many of these cases, there is a natural tendency to call it the 5 hair, but that 5 does not make this decided

jump in position is well evidenced by *Eretmapodites* where both 4 and 5 are easily named by examining the segments on either side. What is believed to be hair 6 is absent from VII of *Mansonia* (*Coquillettidia*).

Hairs 7 (the 1 hair of authors) and 8 (the A hair of authors) form the dorso-lateral hair group and are external to the 5–6 group. Hair 8 is distinct on segments II–VI because of its constant appearance and position. It may range from almost a microspine as in *Culex* to a prominent well-developed spine as in *Anopheles*. Although usually lateral, it may be either dorsal or ventral (on mounted skins at least). The nature of 8 changes markedly on VII where it assumes a similar appearance (usually a multiple plumose hair) to 8 on the modified segment VIII. Hair 7 is internal to 8 and although showing many different forms and degrees of development throughout the subfamily, is quite uniform in position and development on segments II–VI of any individual. As with 8 it usually undergoes a marked change of appearance, and frequently also of position, on VII. In some genera, hair 7 occurs in a ventral position (on mounted skins) on the more posterior segments. Probably what is hair 7 is missing from VII of *Sabethes*.

Hairs 9 and 10 form a ventro-lateral hair group of which hair 9 is the most anterior (except segments III–IV of *Harpagomyia*), and frequently the lesser developed. Hair 9 is distinctly dorsal on the more posterior segments in some genera.

Hairs 11 and 12 form a ventro-sublateral group and lie internal to 9 and 10, and usually also posterior to them. The selection of hair 11 from 12 is not always definitely possible. In general, however, hair 12 is smaller and simpler than 11, and except on segment VI, and frequently VII, is internal to 11. Hair 12 is missing from III–V of *Mansonia* (*Mansonioides*) and is represented by only a hairless setal ring on III–VII of *Mansonia* (*Coquillettidia*).

Hair 13 is similar to hair 1 in being a very uniform microseta. It is situated at the extreme anterior midline of the ventral surface (except in *Trichoprosopon*, where it is associated with the 11–12 group), and is the only pupal seta in which the two members of the pair are intimately associated. In being present or absent, this hair is the most variable of all the abdominal hairs. In the material studied for this project, it was found absent as follows: on segments III–VII in *Sabethes*, *Wyeomyia*, *Phoniomyia*, *Harpagomyia*, *Topomyia*, and *Limatus;* on segment III in *Deinocerites*, *Culiseta*, *Zeugnomyia*, *Orthopodomyia*, *Uranotaenia*, and *Eretmapodites;* on segments III–V in *Megarhinus;* and on VI–VII of *Trichoprosopon* and *Opifex*.

The above comments and data on the setae of the unmodified abdominal segments apply, of course, only to the species examined by us. It is extremely doubtful whether in all cases these modifications represent generic or subgeneric characters.

Modified Segments. Segment I, although possessing a remarkably constant pattern throughout the subfamily, is a highly modified segment. This modification is probably due in part to the proximity of the segment to the water surface and also to the cephalothorax. The sternal sclerotization is completely absent, as are also the ventral setae. Hair 1 is also absent. Hair 2 is usually strikingly developed into a large plumose hair (the float hair or dendritic tuft; not illustrated in detail on most of the figures). The postero-lateral two-thirds of this segment is usually membranous with a sclerotized transverse bar (may be mesally discontinuous with the remainder of the sclerotized portion of the segment in some genera) on its surface. Hair 2 arises on the membrane (on the sclerotized portion in *Mansonia*, however) in a notched portion of the sclerotized tergum and near the medial end of the transverse bar. Hairs 3 and 4 are in the anterior submedian position, 3 usually being distinctly smaller than 4. The 5 and 6 hairs form a definite group antero-laterally, 5 usually distinctly larger than 6. In cases where 5 and 6 are

indistinguishable on the criterion of development, it is deemed advisable to treat them together merely as the 5–6 group and not independently. A hair is associated with the 7–8 group on I that is interpreted to be the normally ventral hair 10 of the unmodified segments. This interpretation, although somewhat arbitrary, is based on the usual presence of a similarly appearing hair on segment II which in some species is disassociated from hairs 7 and 8 sufficiently to be in the normal ventral position of hair 10 (*Limatus* for example). Hair 2 is reduced to an ordinary hair in *Mansonia, Ficalbia,* and *Opifex.*

Segment II is modified in varying but lesser degrees along the same lines as segment I. This modification probably arises as a result of its position at the water surface in the living pupa along with segment I. For example, hair 2 is frequently distinctly mesad of its usual position and takes on an appearance somewhat similar to hair 2 on segment I (*Deinocerites* for example); and in some anophelines a large posterior membranous area similar to that on I is present. Also, as previously pointed out, the hair arrangement of segment II is obviously similar to that on I. Hair 10 is the only ventral hair consistently present on segment II, and it is as often dorsal as ventral. Hairs 9, 11, and 12 may be entirely absent (as in *Bironella*), or present in any combination of one or more (for example, all present in *Limatus*). Hair 13 was found on this segment only in *Tripteroides.*

Segment VIII, although variously modified in shape, has a markedly constant pattern of 4 setae. Hair 1 is always present, although sometimes more posteriorly than is normal on the unmodified segments. Hair 13, which was found in all genera except *Megarhinus,* is nearly always more laterally placed than on the preceding segments. Although the homology of the hair on the postero-lateral angle of the segment is usually clear from a comparison with segment VII, enough exceptions were found (see *Ficalbia* and *Uranotaenia*) to make it necessary to name it arbitrarily (hair 8). In the same manner and because of this strong similarity between the postero-lateral hairs of VII and VIII, hair 8 has sometimes been selected on VII by appearance, without regard to position. It seems quite likely that in some genera, the postero-lateral hair of VIII is actually hair 7, but to attempt to delineate these cases would mean having some situations in which no decision could be made at all; consequently, it was felt that an arbitrary decision was the best course. The slighter hair located mesad of 8 and overhanging the base of the paddle was also arbitrarily named (hair 5), since it too may resemble various hairs on segment VII in the different genera.

Segment IX. Medially produced from the posterior margin of segment VIII is a dorsal flap which is designated as the anal flap. This and the paddles (following Edwards, 1941) are regarded as the remnants of segment IX. In a number of the genera a small lateral hair is present on the anal flap and it has been arbitrarily designated as hair 1 by us. The paddle hair has arbitrarily been named hair 8 (absent in *Megarhinus,* all of the sabethines, *Mansonia,* and *Ficalbia*). In the anophelines, a ventral accessory paddle hair is present and in *Uranotaenia* (*Pseudoficalbia*) and *Culex* a medial terminal accessory paddle hair occurs. In both positions, this accessory hair has arbitrarily been designated as hair 7.

Segment X. The genital sac is regarded as part of segment X. In *Megarhinus,* it bears a prominent branched hair which has arbitrarily been designated as hair 8 (genital hair).

SUMMARY

A comparative study of the chaetotaxy of the mosquito pupa was made by examining and illustrating the pupa of at least one species each of 28 of the 30 known mosquito genera.

No means of establishing homologies between the cephalothoracic and ab-

dominal hairs were discovered. The 12 pairs of cephalothoracic setae were designated with arabic numerals (1–12), beginning with the most anterior group and naming laterally and posteriorly the hairs in each succeeding group.

In general, it was found possible on the basis of similarities in position and development to establish homologies for the setae of abdominal segments I to VII, both between the hairs of each segment of the individual and also between the hairs of equivalent segments of the different genera studied. However, segments VIII to X were found to be so extensivly modified that no means of determining the homologies of their setae with those of the other abdominal segments were discovered.

In establishing the relationships between the hairs of segments I to VII, it was found that by proceeding in either direction from VI hair relationships could usually be more readily recognized and justified than by beginning with any other segment.

By the use of this method for determining hair relationships, a nomenclature of arabic numerals (0–13) was applied to the setae and hairless setal rings of segments I to VII, beginning at the dorsal midline and extending laterally and ventrally to the ventral midline. The hairs of segments VIII to X were arbitrarily named with arabic numerals.

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Of the pupal collection assembled for this investigation, 15 of the species were from the collection made in the Pacific in 1945 under the auspices of U. S. Naval Medical Research Unit 2 by L. E. Rozeboom, K. L. Knight, and J. L. Laffoon. These specimens are all to be deposited in the U. S. National Museum. The remainder of the material was made available to us through the generosity of Alan Stone, U. S. National Museum; P. F. Mattingly, British Museum (Natural History); H. R. Roberts, Academy of Natural Sciences of Philadelphia; L. J. Dumbleton, Dept. Scientific and Industrial Research, Wellington, New Zealand; H. R. Dodge, U. S. Public Heath Service; and L. E. Rozeboom, Johns Hopkins University. To these people we wish to express our sincerest appreciation.

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EXPLANATION OF FIGURES

The cephalothoracic drawings are of skins opened along the dorsal midline with the outer surface up. The ventral midline is median in the complete drawing and at the right in the incomplete figures. All of the metanotal and abdominal drawings show the ventral surface on the left and the dorsal surface on the right. In each case the first segment shown is the metanotum.

The systematic arrangement used in the following pages is, except for the treatment of the sabethines, according to Edwards (1932). Following Lane and Cerqueira (1942) the sabethines have been raised to tribal rank and certain generic changes adopted.

Unless otherwise stated, the specimens used for the drawings are all deposited in the U. S. National Museum, Washington, D. C.

Metanotal and Abdominal Figures.

Anophelini

Figure 1. *Chagasia bathanus* (Dyar). Panama.

" 2. *Bironella* (*Brugella*) *hollandi* Taylor. Poha River, Guadalcanal Island, Solomons (L. E. Rozeboom). Ex stream margin.

" 3. *Anopheles* (*Myzomyia*) *farauti* Laveran. Hollandia, Dutch New Guinea (H. Hoogstraal).

Megarhinini

" 4. *Megarhinus amboinensis* (Doleschall). Group *Toxorhynchites*. Lake Sentani, Hollandia, Dutch New Guinea (K. L. Knight). Ex coconut shell.

Sabethini

" 5. *Trichoprosopon* (*Trichoprosopon*) *compressum* Lutz. Rio de Janeiro, Brazil (L. Whitman).

" 6. *Tripteroides* (*Mimeteomyia*) *caledonica* (Edwards). Espiritu Santo Island, New Hebrides (J. L. Laffoon). Ex tree hole.

" 7. *Sabethes* (*Sabethinus*) *aurescens* Lutz. Distrito Federal, Rio de Janeiro, Brazil (L. Whitman).

" 8. *Wyeomyia* (*Wyeomyia*) *lutzi* (Lima). Distrito Federal, Rio de Janeiro, Brazil (L. Whitman).

" 9. *Phoniomyia edwardsi* Lane and Cerqueira. Paratype slide. Distrito Federal, Rio de Janeiro, Brazil (L. Whitman).

" 10. *Limatus durhamii* Theobald. Villavicencio, Colombia (L. E. Rozeboom). Specimen in collection of School of Hygiene and Public Health, Johns Hopkins University.

" 11. *Topomyia barbus* Baisas. Tacloban, Leyte Island, Philippines (H. R. Roberts). Ex abaca axil. Specimen in collection of Academy of Natural Sciences of Philadelphia.

" 12. *Harpagomyia genurostris* (Leicester). Calotons (Basey River, Basey Municipality), Samar Island, Philippines (M. J. MacMillan). Ex taro axil.

Culicini: Uranotaenia Group

" 13. *Hodgesia spoliata* Edwards. Lake Sentani, Hollandia, Dutch New Guinea (L. E. Rozeboom). Ex open swamp.

" 14. *Zeugnomyia lawtoni* Baisas. Barugwan River, Tacloban, Leyte Island, Philippines (H. R. Roberts). Ex water in large dead leaves. Specimen in collection of Academy of Natural Sciences of Philadelphia.

" 15. *Uranotaenia argyrotarsis* Leicester. Group *Uranotaenia*. Irahuan River Valley, Palawan Island, Philippines (J. L. Laffoon). Ex ground pool.

" 16. *Uranotaenia nigerrima* Taylor. Group *Pseudoficalbia*. Lake Sentani, Hollandia, Dutch New Guinea (K. L. Knight). Ex fallen sago palm frond.

Culicini: Culiseta-Mansonia Group

" 17. *Culiseta* (*Culiseta*) *incidens* (Thomson). California.
" 18. *Orthopodomyia mcgregori* (Banks). Group *Orthopodomyia*. Irahuan River Valley, Palawan Island, Philippines (D. R. Johnson). Ex treehole.
" 19. *Ficalbia* (*Etorleptiomyia*) *elegans* (Taylor). Guadacanal Island, Solomons (L. E. Rozeboom). Ex wooded swamp.
" 20. *Aedeomyia catasticta* Knab. Iwahig, Palawan Island, Philippines (J. L. Laffoon). Ex irrigation reservoir.
" 21. *Mansonia* (*Coquillettidia*) *xanthogaster* (Edwards). Brigstocke Point, Espiritu Santo Island, New Hebrides (L. E. Rozeboom). Ex open swamp.
" 22. *Mansonia* (*Mansonioides*) *uniformis* (Theobald). Tacloban, Leyte Island, Philippines (K. L. Knight). Ex open swamp.

Culicini: Aedes Group

" 23. *Psorophora* (*Psorophora*) *ciliata* (Fabricius). Ithaca, N. Y. (O. A. Johannsen).
" 24. *Opifex fuscus* Hutton. Wellington, New Zealand (G. Hudson).
" 25. *Aedes* (*Mucidus*) *ferinus* Knight. Holotype slide. San Ramon (Penal Farm), City of Zamboanga Province, Mindanao Island, Philippines (J. L. Laffoon, K. L. Knight). Ex ground pool.
" 26. *Haemagogus* (*Haemagogus*) *capricornii* Lutz. Serra da Cantareira, San Paulo, Brazil (Lerio Gomes).
" 27. *Eretmapodites leucopus productus* Edwards. Bwamba, Uganda (A. J. Haddow).
" 28. *Armigeres* (*Armigeres*) *malayi* (Theobald). Ducong (Basey River, Basey Municipality), Samar Island, Philippines (M. J. MacMillan). Ex coconut shell.

Culicini: Culex Group

" 29. *Culex* (*Lutzia*) *halifaxii* Theobald. Sohoton Springs (Basey River, Basey Municipality), Samar Island, Philippines (K. L. Knight). Ex rock pool.
" 30. *Deinocerites spanius* (Dyar and Knab). Group C-*Dinamamesus*. Brownsville, Texas (H. R. Dodge). Ex crab hole.

Cephalothoracic Figures.

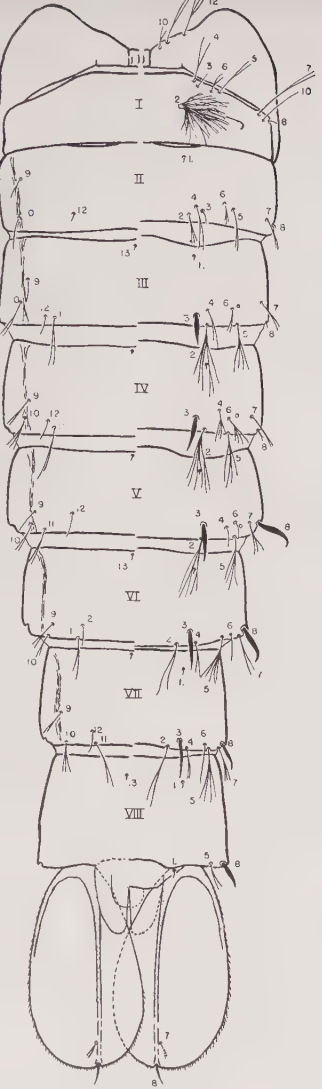
Figure 31. *Anopheles* (*Myzomia*) *farauti* Laveran. Espiritu Santo Island, New Hebrides (K. L. Knight).
" 32. *Culex binigrolineatus* Knight and Rozeboom. Lake Sentani, Hollandia, Dutch New Guinea (K. L. Knight). Ex sago palm axil.
" 33. *Aedes* (*Finlaya*) *niveus* (Ludlow). Olongapo (Subic Bay), Zambales Providence, Luzon Island, Philippines (L. E. Rozeboom). Ex tree hole.
" 34. *Megarhinus horei* Gordon and Evans. Group *Megarhinus*. Yurimena, Colombia (M. Bates, L. E. Rozeboom). Ex axil of Ravenala palm. Specimen in collection of School of Hygiene and Public Health, Johns Hopkins University.
" 35. *Uranotaenia geometrica* Theobald. Group *Uranotaenia*. Villavicencio, Colombia (L. E. Rozeboom). Specimen in collection of School of Hygiene and Public Health, Johns Hopkins University.

Observations on Oribatid Mite Vectors of *Moniezia expansa* on Pastures, with a Report of Several New Vectors from the United States

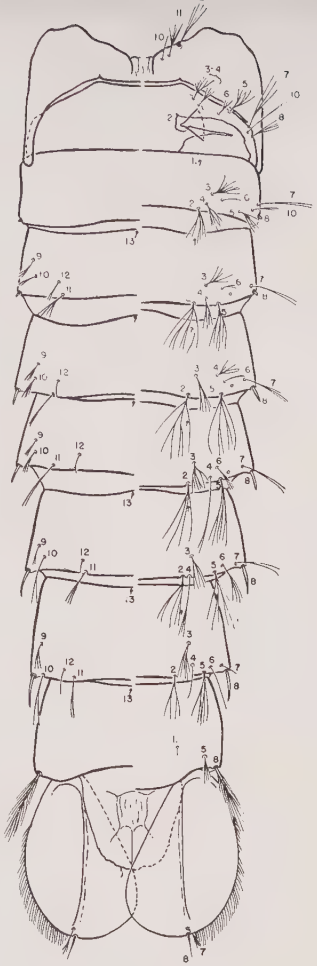
K. C. KATES AND C. E. RUNKEL

U. S. Bureau of Animal Industry

Since the announcement by Stunkard (1937) that oribatid mites serve as intermediate hosts of the common sheep tapeworm, *Moniezia expansa*, eight additional species of anoplocephaline cestodes that parasitize mammals have found



1. CHAGASIA



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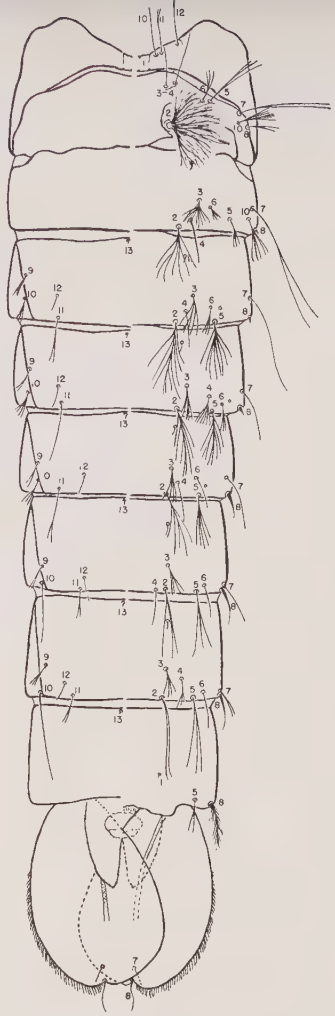

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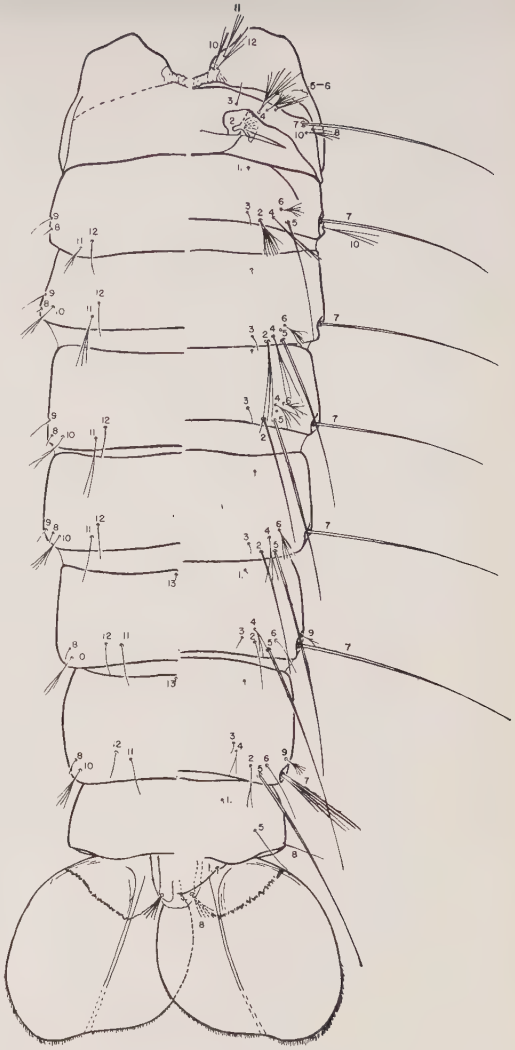

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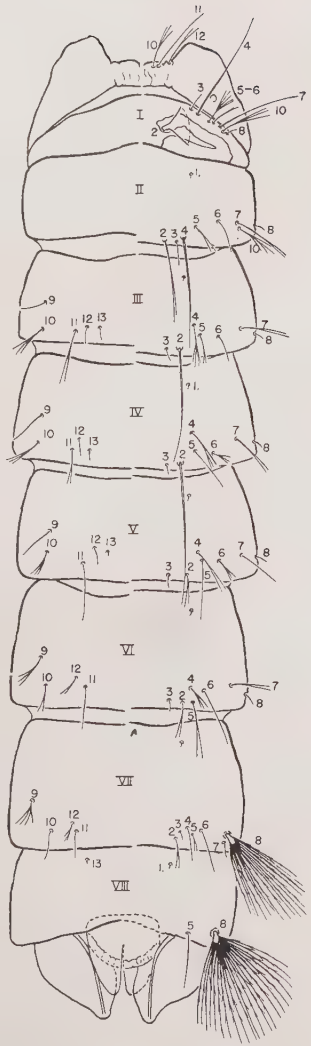

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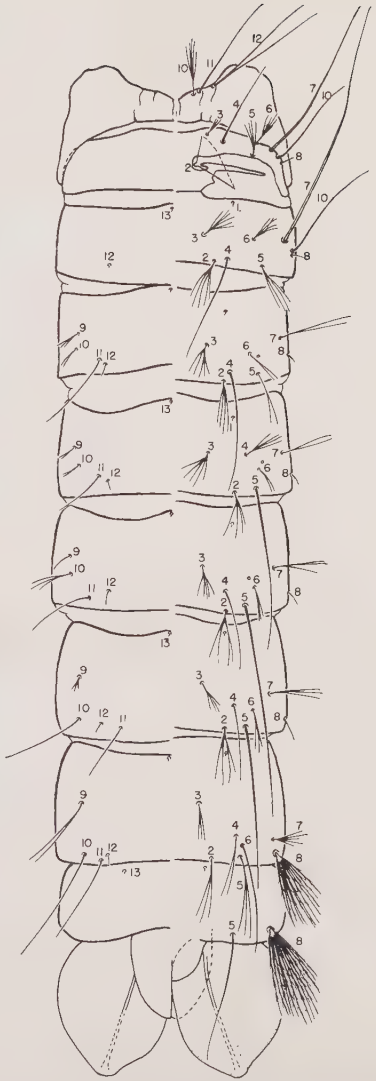

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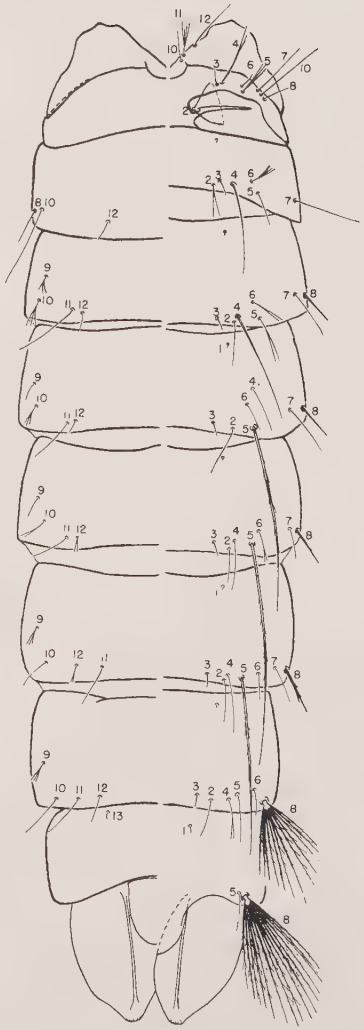

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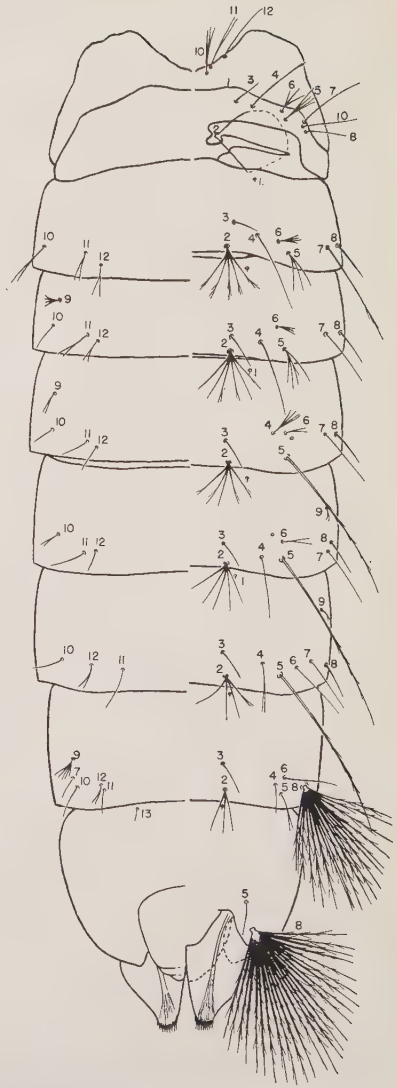
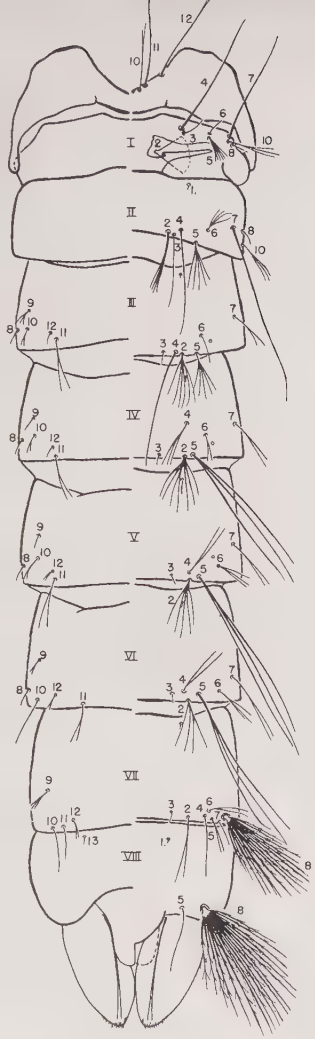


8. WYEOMYIA



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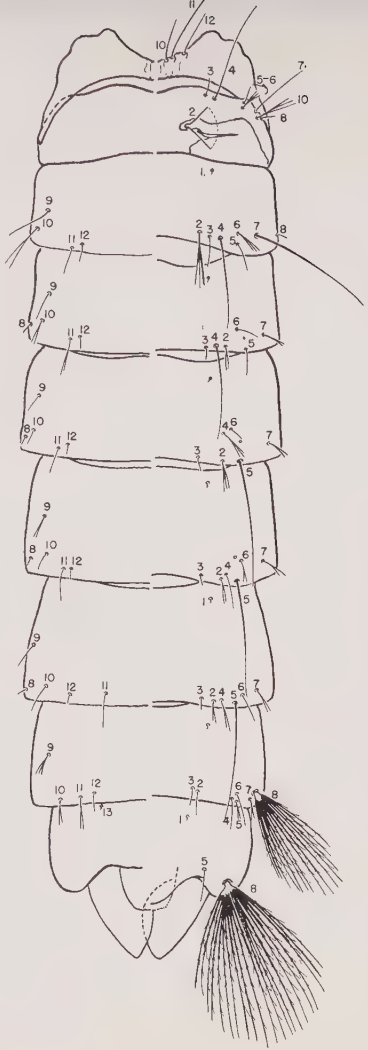

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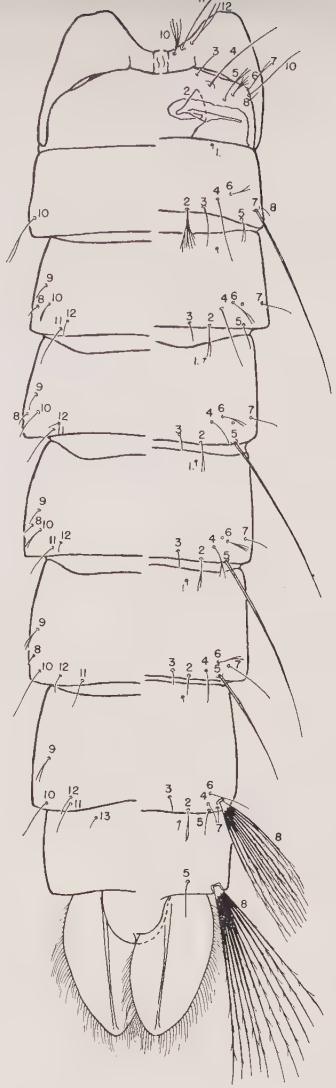

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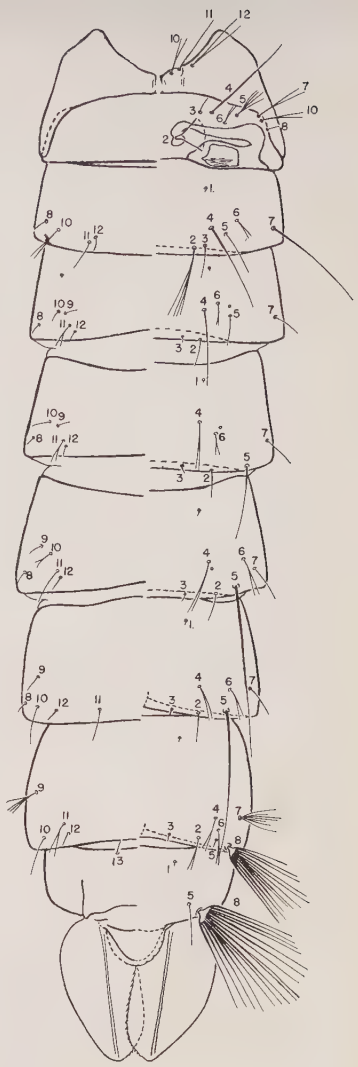

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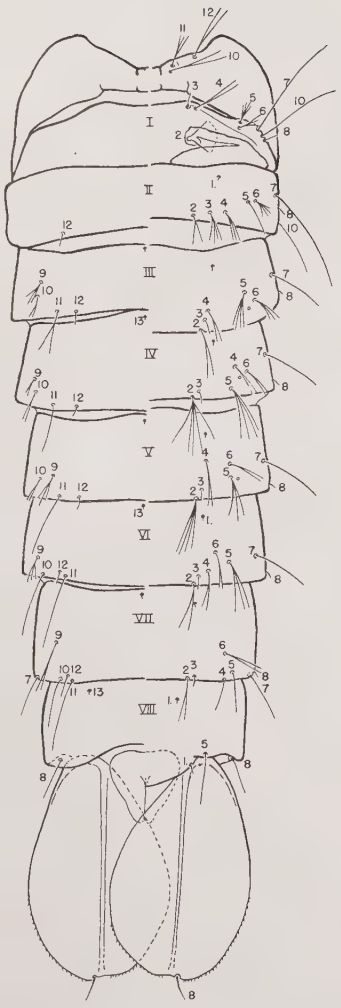

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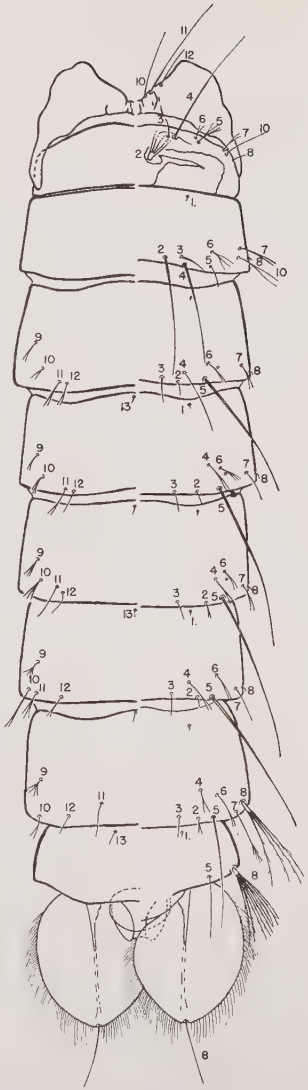

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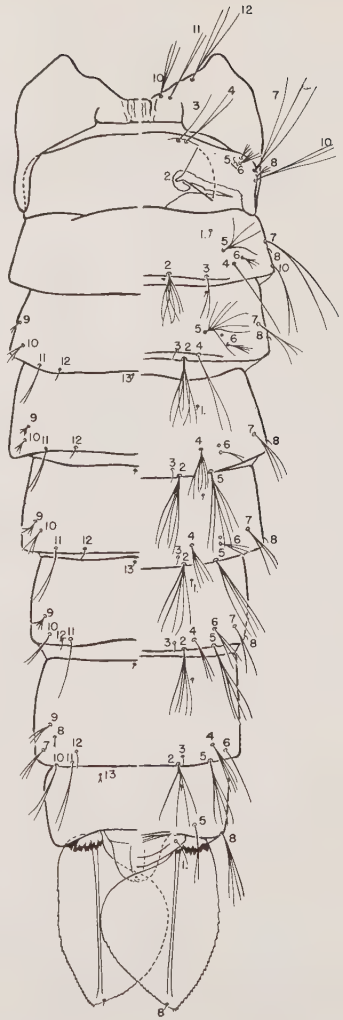
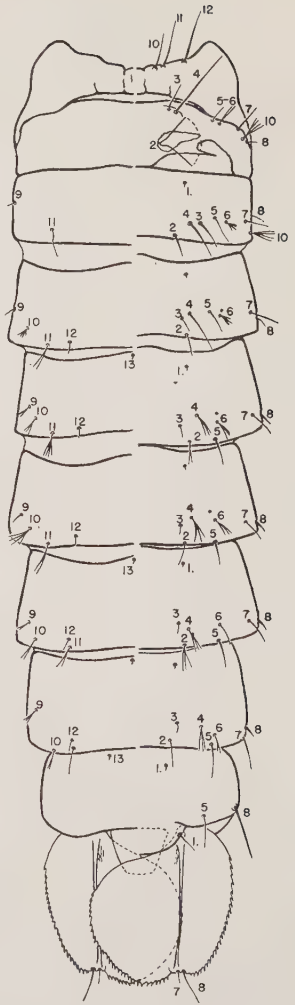


15. URANOTAENIA (URAN.)



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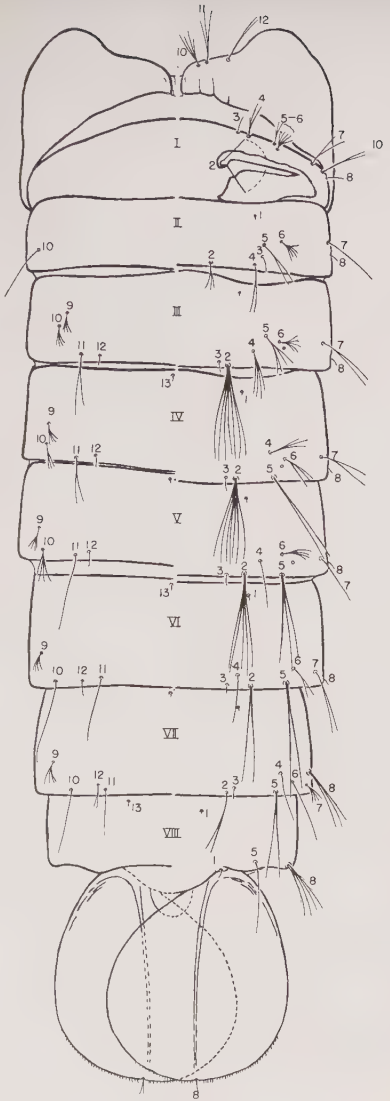

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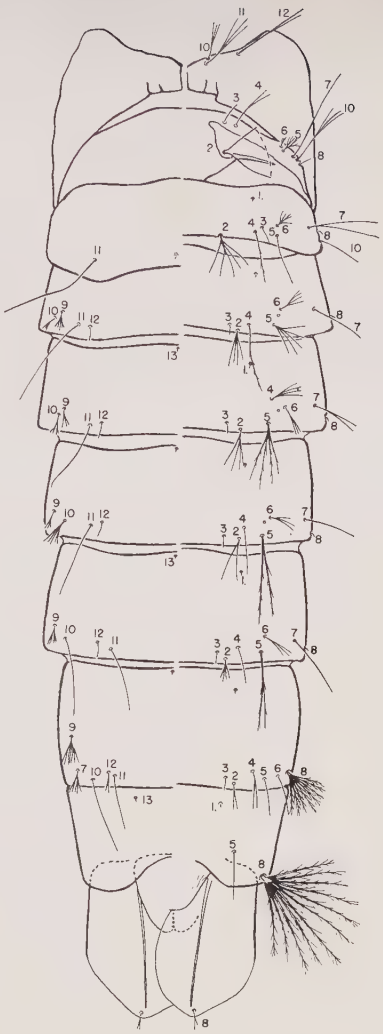

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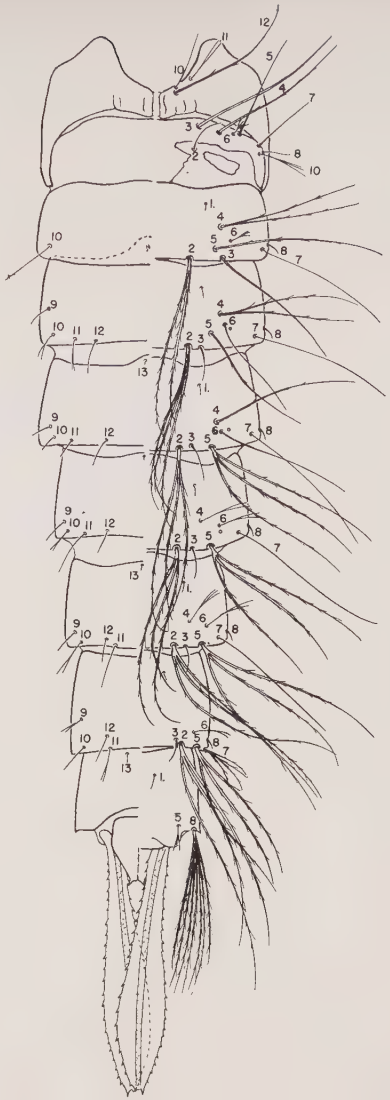

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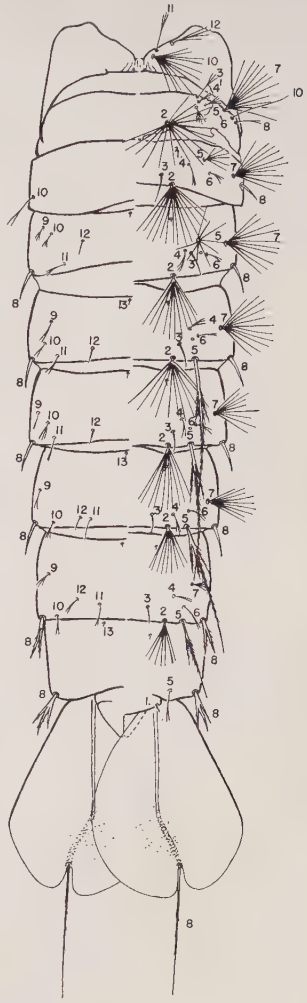

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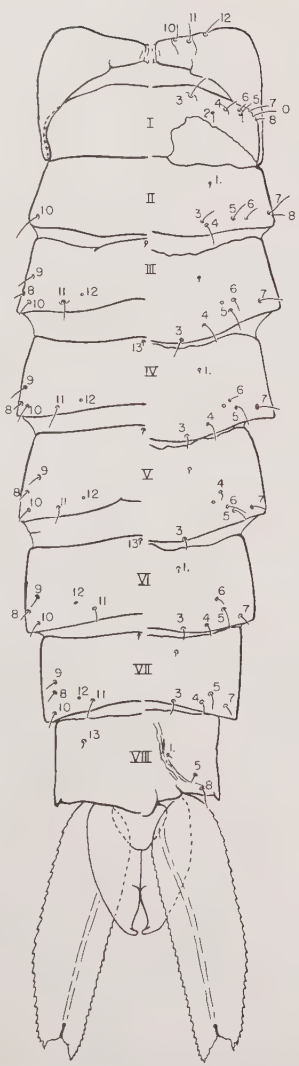
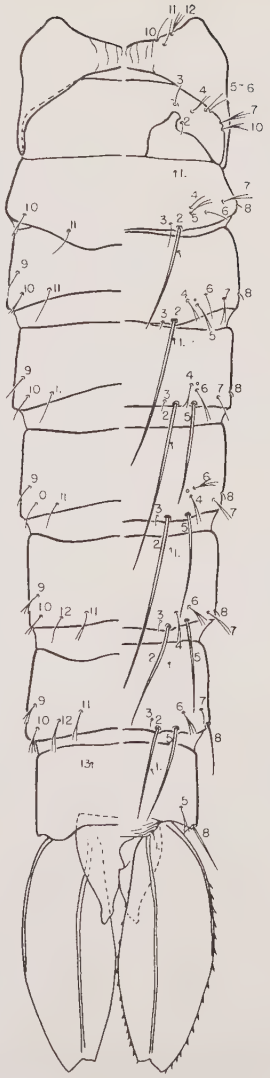


21. MANSONIA (COQ)



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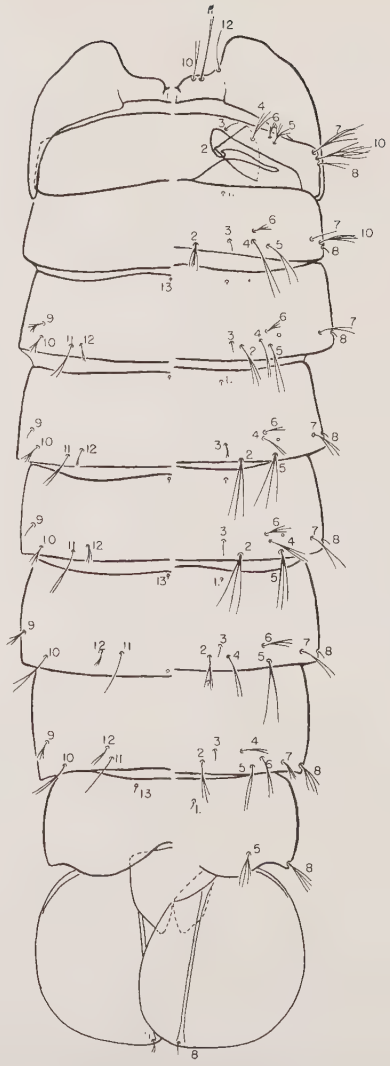

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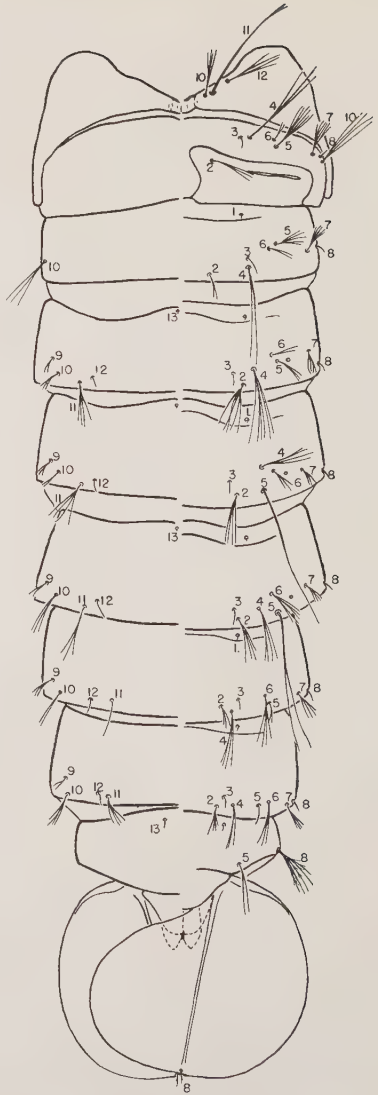
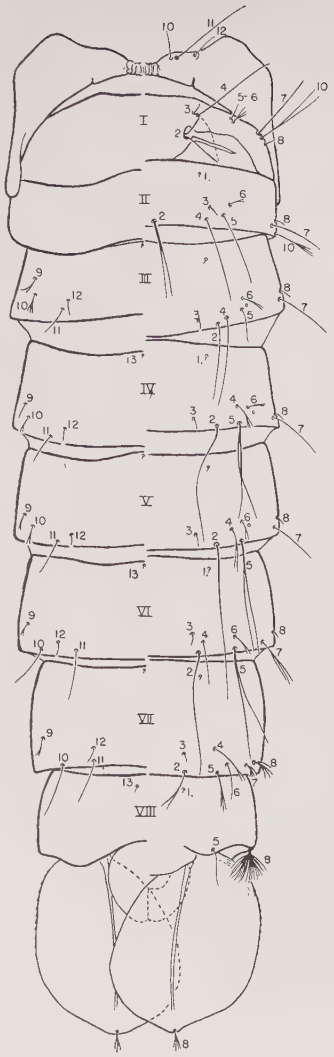


24. OPIFEX



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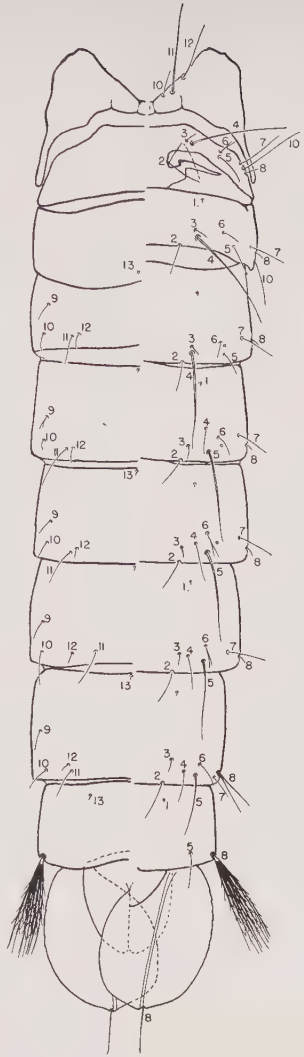

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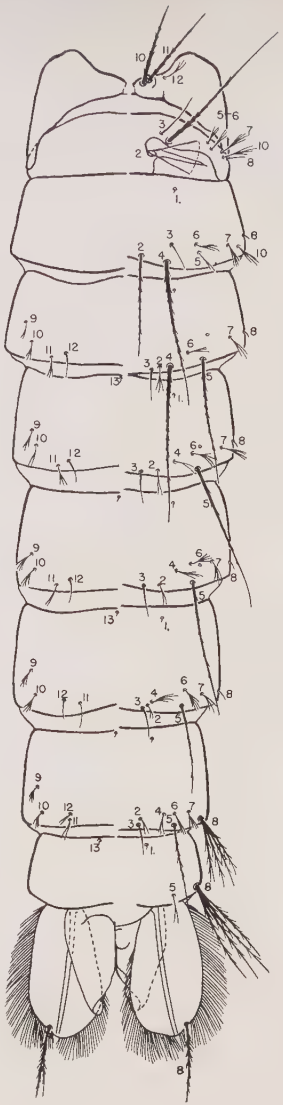
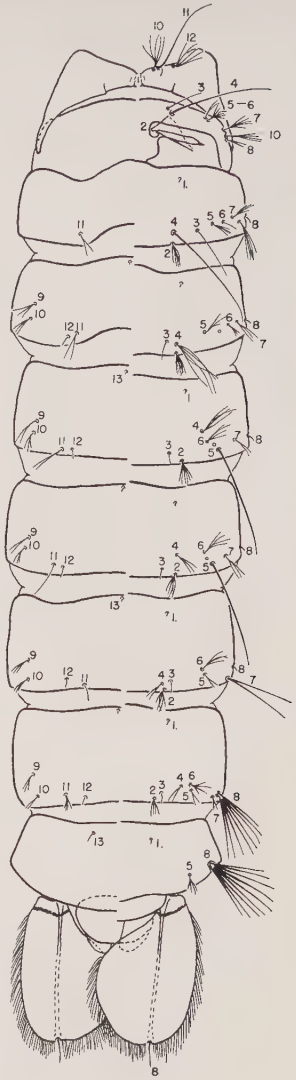
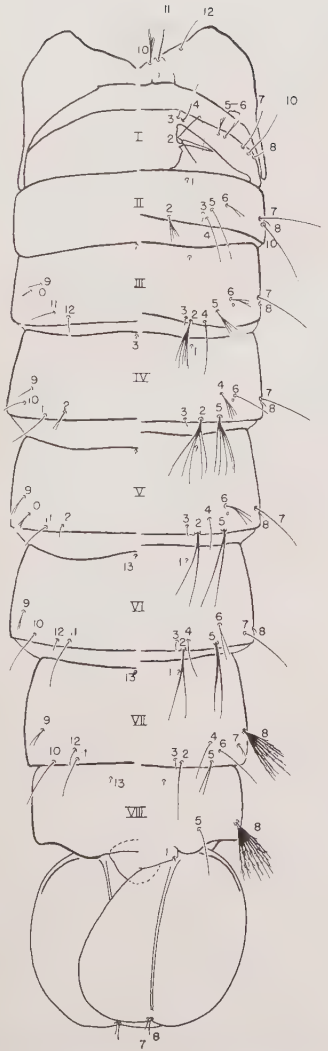


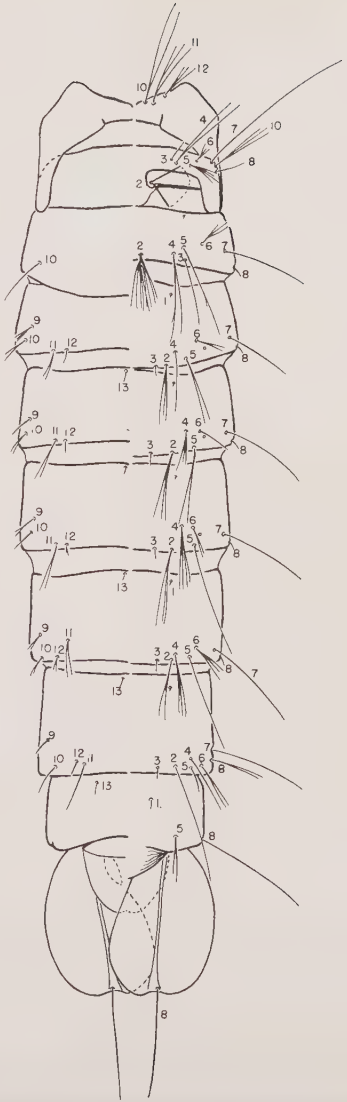
27. ERETMAPODITES



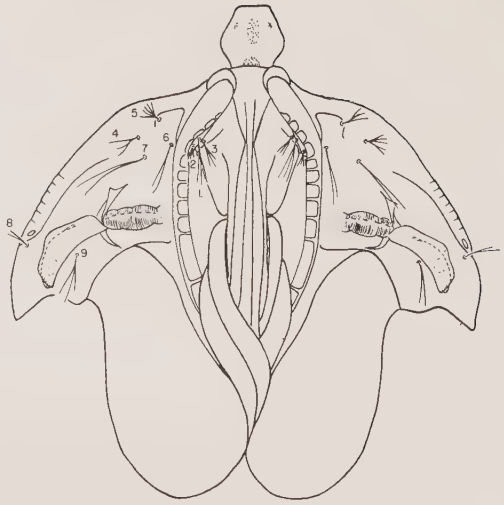
28. ARMIGERES



29. CULEX



30. DEINOCERITES



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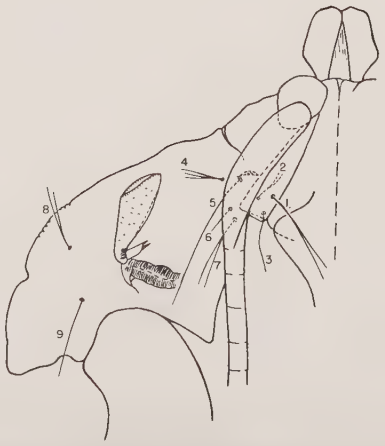

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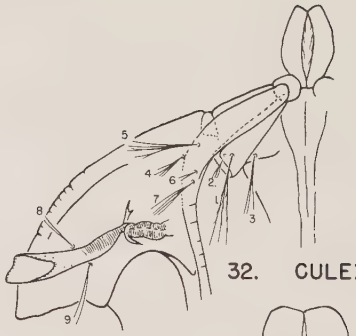

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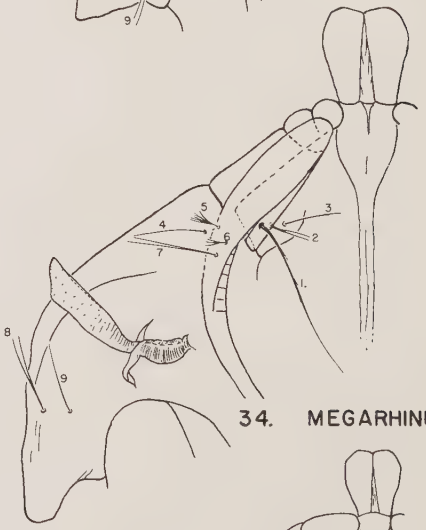
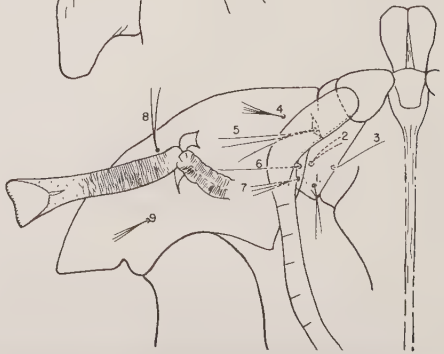


34. MEGARHINUS



35. URANOTAENIA

to undergo larval development in these invertebrates, namely, *Bertiella studeri* of primates reported by Stunkard (1940), *Cittotaenia ctenoides* and *C. denticulata* of rabbits reported by Stunkard (1941), *Anoplocephala perfoliata, A. magna* and *Paranoplocephala mamillana* of equines reported by Bashkirova (1941), *Moniezia benedeni* of ruminants reported by Potemkina (1944a), and *Thysaniezia giardi* (syns. *Helictometra giardi, Thysaniezia ovilla*) of sheep reported by Potemkina (1944b). Confirmatory studies on oribatid-mite transmission of *M. expansa* have been reported by Stoll (1938), Krull (1939a), Shorb (1939) and Potemkina (1941).

A review of the literature on transmission of these tapeworms shows that development of their larval stages may occur in a number of genera and species of more than a half dozen families of oribatid mites. The major part of the life history work has been done by feeding tapeworm eggs to mites in the laboratory. Little information has been available heretofore concerning (1) the mite vectors of anoplocephaline cestodes under natural pasture or other conditions, (2) the species of mites inhabiting different environments and localities, (3) bionomics of the mites, and (4) incidence of cysticercoid infections of mites under natural conditions.

In order to carry out experiments with *M. expansa* and to provide a reliable source of cysticercoids, extensive collections of orbatid mites were made during 1946 and 1947, principally at the Agricultural Research Center, Beltsville, Md., from which cysticercoids were obtained by dissection. The mite collections were made from two permanent sheep pastures—one at Beltsville, Md., and one at Newell, S. Dak.—and one experimentally inoculated pasture plot at Beltsville, Md. The results of these studies are reported in this paper, which contains observations (1) on the systematic position, distribution, and ecology of six species of mites found to be vectors of *M. expansa* under natural conditions on pastures, four of these being new intermediate hosts and one previously unreported from the United States, and (2) cysticercoid infection rates and capacities of these mites.

The writers gratefully acknowledge the assistance of Dr. R. T. Habermann, who collected mites from an irrigated sheep pasture at Newell, S. Dak., and sent them to us for study, and the considerable aid in identification of oribatid mites given us by Dr. E. W. Baker, of the U. S. Bureau of Entomology and Plant Quarantine.

MATERIALS AND METHODS

Sources of mite collections.—Oribatid mites reported in this paper as vectors of *M. expansa* were collected from the following places: (1) A 12-acre permanent sheep pasture at the Agricultural Research Center, Beltsville, Md.; (2) a 12-acre, irrigated, permanent pasture at Newell, S. Dak.; (3) a small pasture plot, 20 by 60 feet, at Beltsville, Md., on which feces containing eggs of *M. expansa* had been placed the previous year. These three collection areas will be designated in the text, respectively, as pastures A and B and pasture plot C. Pasture A was an excellent bluegrass pasture with very little shade. It had been grazed from year to year by sheep for a total period of about 22 years. Pasture B was comparable to pasture A and had been used in a similar manner; this pasture was irrigated. These two permanent pastures, therefore, are roughly comparable as to size and use, but were located in widely separated parts of the country and subjected to different climatic conditions. Pasture plot C was fenced off from a small experimental pasture, one side of the plot being adjacent to an undeveloped wooded area, and the other three sides adjacent to the small experimental pasture. Three large pine trees cast considerable shade over the 1200 square feet area of this plot. Early in 1946 preliminary collections of oribatid mites were made from

turf samples taken from this plot, and three species of galumnid mites and several species of non-galumnid mites were recovered. Several thousand of these mites were examined for cysticercoids and found to be uninfected; this plot had not been grazed by sheep for about four years. The plot, therefore, was covered with a thick layer of sheep droppings, containing eggs and proglottids of *M. expansa*, during the summer and fall of 1946. The concentration of tapeworm eggs on this plot was much greater than that found on a comparable area of permanent sheep pasture. The first cysticercoids were obtained from mites collected from this plot in November 1946. Extensive collections were begun in February 1947 and continued during the spring and summer of that year.

Collection of oribatid mites from pasture turf.—Various reports on soil fauna show that oribatid mites are recovered in the greatest numbers from the humus layer of the soil where the organic content is very high [Willmann (1931), Jacot (1936 & 1940b), Soldatova (1945), Pearse (1946)]. The writers determined, by preliminary collections or oribatid mites from different depths of pasture soils, that the majority are found in the first inch of turf, consisting of the surface grass, humus, grass roots and included soil. Throughout our collections, therefore, turf samples, one square foot in size and approximately one inch thick, were cut from the collection areas.

Two methods of collecting oribatid mites from turf were tried, namely, (1) the "drying cone" or modified Berlese funnel method, slightly but not essentially modified from that described by Tragardh (1933), Jacot (1936), Potemkina (1941) and Starling (1944), and (2) the washing-screening-flotation method of Krull (1939b) somewhat simplified and applied to turf instead of grass samples. Trial collections, employing both methods, were made and approximately similar quantities and species of mites were obtained from equivalent turf samples. The drying cone method, however, was employed almost exclusively in our collections, because it required much less time and labor to recover mites from equivalent quantities of turf and caused less injury to the mites.

A battery of drying cones was employed, each cone having a top diameter of 18 inches and a turf capacity of one square foot. A 200-watt electric light bulb, suspended about three inches over the turf in the cones, provided the heat for gradual drying of the turf, and light for the downward migration of the negatively phototropic mites. The fixed 20-mesh screen, placed four inches from the open top of each funnel, was overlaid with a double layer of gauze and the turf samples placed, grass side down, on the gauze. By this procedure only oribatid mites and other small soil invertebrates were recovered in the $\frac{1}{2}$-pint fruit jars, the lids of which were soldered to the cone vents. About $\frac{1}{4}$ inch of water was added to the collecting jars in which the mites, migrating down the cones, were trapped. Maximum recovery of oribatid mites was obtained after a 48-hour exposure of turf samples in the drying cones. Thereafter, the collecting bottles were unscrewed from the drying cones, the mites removed from the water surface with pointed wood applicators, placed in 50-cc. weighing bottles containing small pieces of filter paper and a few drops of water, and the bottles tightly stoppered. Oribatid mites may be stored in this manner for several weeks.

Permanent mounting of mites for study and identification.—Permanent mounts of oribatid mites were made by first killing them in 70 per cent alcohol, and then mounting them directly on a glass slide in a polyvinyl alcohol–lactic acid medium (Downs, 1943). Living mites may be mounted in the same manner without preliminary treatment with alcohol. A very satisfactory combined mounting and clearing medium was made by combining 75 parts of a syrupy, aqueous solution of polyvinyl alcohol with 25 parts of lactic acid. Mites mounted by this method were clear enough for study in a few hours to several days, depending

upon the opacity of the specimens. Although it is possible to study adult oribatid mites of all species mounted in this manner, no completely satisfactory method of clearing adult specimens of the more opaque species, such as galumnid mites, was discovered. Excellent preparations, however, were made of opaque species by mounting young specimens having all adult features except the deep brown color in the exoskeleton. Some interesting preparations were made of less opaque species of mites containing cysticercoids (Fig. 1, A, B, C). Once an infected mite is mounted in the plastic-lactic acid medium and has cleared for a few hours, cysticercoids can be seen clearly, but exposure for long periods gradually distorts the cysticercoids and renders them too transparent.

Dissection of mites for cysticercoids.—Once the species of mites from the collection areas was determined by study of mounted specimens, it was possible to separate them as to species while alive by use of a dissecting microscope. Any mites of questionable identity were mounted and studied under higher magnification. Once the species of mites were separated, groups of 10 each were placed in two small drops of physiologic saline on a slide, each group covered gently with a small coverslip, and the slide placed on the stage of a dissecting microscope on which was concentrated a strong beam of light from above. The mites were then crushed by gentle pressure on the coverslip by a dissecting needle and the number of cysticercoids in each infected mite determined and recorded. The rather brittle mites can be crushed, without apparent damage to the cysticercoids, by controlled pressure on the coverslip. Thereafter, the coverslip was gently removed, cysticeroids concentrated in one group in the center of the drop, and then removed with a capillary pipette to a small watch glass. Thus, it was possible to determine the percentage of infected mites of each species, the number of cysticercoids in each specimen and, at the same time, have cysticercoids available for experimental infection of lambs. A record was made for each unit mite collection (usually five to seven square feet of turf) of (1) total number of mites of each species known, or determined to be, vectors of *M. expansa*, (2) number of mites with cysticercoids, and (3) number of cysticercoids in each infected mite.

The consistently successful results of experimental feeding of cysticercoids, so collected, to lambs, and the fact that collections of infected mites were made from turf either of an experimentally inoculated pasture plot or from permanent pastures grazed by infected sheep for many years, showed that these cysticercoids were those of *M. expansa*.

SYSTEMATIC POSITION, DISTRIBUTION AND ECOLOGY

Systematic Position

Because of the difficulties encountered in accurate identification of all oribatid mites collected from soil samples, we restricted our taxonomic efforts mainly to those species from which cysticercoids were obtained. Other species of mites were collected in varying numbers, as reported by Krull (1939b), but no detailed records of these were kept. Descriptions and figures of American oribatid mites are available for only a fraction of the total species. Of the six species of mites in our collections found to contain *M. expansa* cysticercoids, one species appeared to be new and has been described by us (Runkel & Kates, 1947), two species were identified from excellent descriptions and figures in the literature, while the remaining three species were identified only after comparison with types or paratypes in the collection of Acarina at the U. S. National Museum; the existing descriptions and figures, if any, of the mites so identified are unsatisfactory for identification purposes. As three of the six species of *Moniezia* vectors upon which this report is based have not been adequately described and figured, although named, in the literature, drawings and photographs are presented in plate I which may be of aid

to others interested in anoplocephaline tapeworm problems of domestic and wild animals. This group of oribatid mites is of particular interest in that it consists of typical members of four families and five genera and illustrates the approximate minimum and maximum development of the pteromorphae of the "winged" or pterogasterine oribatids.

The mites (Fig. 1) were identified as follows: *Galumna virginiensis* Jacot, 1929 from pasture A at Beltsville, Md.; *Scheloribates laevigatus* (Koch, 1836) from pasture B at Newell, S. Dak.; *Galumna emarginatum* (Banks, 1895), *Oribatula minuta* (Ewing, 1909), *Peloribates curtipilus* Jacot, 1937, and *Protoschelobates seghettii* Runkel and Kates, 1947, in addition to *G. virginiensis,* from pasture plot C at Beltsville, Md.

The species of oribatid mites previously reported as vectors of *M. expansa* belong, with one exception, to the genus *Galumna,* family Galumnidae. This report adds to the list of vectors four species, belonging to three genera and two families, namely, *Galumna virginiensis* (Galumnidae), *Oribatula minuta* (Oribatulidae), *Peloribates curtipilus* (Haplozetidae), and *Protoschelobates seghettii* (Scheloribatidae). In addition *Scheloribates laevigatus* (Scheloribatidae), which has been reported as a vector of this tapeworm in Russia (Potemkina, 1941), but had not previously been reported from the United States, was collected from the pasture at Newell, S. Dak., and found to harbor cysticercoids of *M. expansa.*

No attempt will be made in this paper to describe the differential characters of the six species of mites, but figures illustrating these characters are presented in Fig. 1 and the references to the literature on this subject are given in the bibliography.

In regard to the structure and taxonomy of these mites we have found the various papers of Jacot particularly helpful, especially for the galumnid mites, and much useful background information was obtained from the monograph of Willmann (1931). Our most satisfactory source of reference was the collection of Acarina, reprints and other publications at the U. S. National Museum and their official custodian, Dr. E. W. Baker.

Distribution and Ecology

Mite collections from pasture A, Beltsville, Md.—Extensive collections of mites were made from turf of this pasture, several being made each month from July 1946 to April 1947, except for the month of March 1947 (Table 1). Only one species of mite, *G. virginiensis* (Fig. 1, F & L), was found to contain cysticercoids. Not only was this species the only vector of *M. expansa* found, but it was also the most numerous of the species in our collections. A total of 52,602 *G. virginiensis* were collected from 395 square feet of turf, an average of about 133 mites per square foot. Usually the turf samples were placed in the drying cones, 5 to 7 square feet at a time, each lot of turf being collected on different days. Considerable variation occurred in the number of mites collected from unit lots of turf. The number varied from 8 to 375 specimens of *G. virginiensis* per square foot, the maximum number being from a September collection. This variation in "take" of mites from different turf samples appeared to be least influenced by seasonal or temperature conditions, since 6 square feet of frozen turf were chopped from the pasture with an axe in January 1947 after an extended period of severe frost and 193 *G. virginiensis* per square foot of turf were collected therefrom. Other winter collections were as high as 334 mites of this species per square foot.

Although it is not possible to determine accurately by the methods employed, the maximum mite populations of a given area of pasture, a minimum estimate of

about 6,000,000 *G. virginiensis* per acre of this pasture may be calculated from the data. It is likely that the actual total pasture population of this species is considerably larger than this estimate indicates.

In addition to *G. virginiensis*, another galumnid mite, *G. curvum* (Ewing, 1907), occurred frequently in our collections. This species is about half the size of *G. virginiensis*, measuring about 0.3 mm. in length, and is one of the smallest known galumnas; no cysticercoids were found in several thousand specimens examined. Several hundred specimens of oribatid mites other than the galumnas were also free of cysticercoids.

Although *G. virginiensis* was recovered in relatively large numbers from pasture turf, it was either absent altogether or present in only small numbers in mite collections made from forest soils or locations other than permanent pasture. The fact that this species is an excellent vector of *M. expansa* and the only one found on this permanent pasture, would indicate that it is more coprophagous than other species taken in the same collections. An open sheep pasture exposed to climatic

TABLE 1.—*Summary of collections of* Galumna virginiensis *from turf of pasture A, Beltsville, Md.*

Month	Square feet of turf	Mites collected	Mites per square foot (average)
1946			
July	145	13,464	92
August	87	15,989	182
September	40	5,289	132
October	25	2,804	112
November	40	4,329	108
December	11	3,127	284
1947			
January	35	5,374	153
February	6	476	79
April	6	1,750	291
Totals	395	52,602	133

conditions of the Beltsville, Md. area and regularly manured with the droppings of grazing animals appears to be an ideal habitat for this species.

Mite collections from pasture B, Newell, S. Dak.—Seven separate mite collections, each from one square foot of turf, were made from this pasture. One collection was taken in July and six in September 1946 from different parts of the pasture. The mites were sent alive to us at Beltsville and arrived in excellent condition. The most abundant mite and only *M. expansa* vector recovered was *S. laevigatus* (Fig. 1, E & K). A number of smaller oribatid mites were also present in the collections, but no cysticercoids were recovered from them. Except for the difference in species and smaller quantity of turf examined, the collection data were similar to those for *G. virginiensis* from the Beltsville, Md., pasture. A total of 1,552 *S. laevigatus* were recovered from the seven square feet of turf, an average of 221 per square foot. The population of this species per acre may be estimated at over 9,000,000, a figure fairly close to the 6,000,000 per acre for *G. virginiensis* at Beltsville.

Mite collections from pasture plot C, Beltsville, Md.—Results of collections from this plot were quite different from those of the two permanent pastures. As previously mentioned, this plot had not been grazed by sheep for over 4 years, and

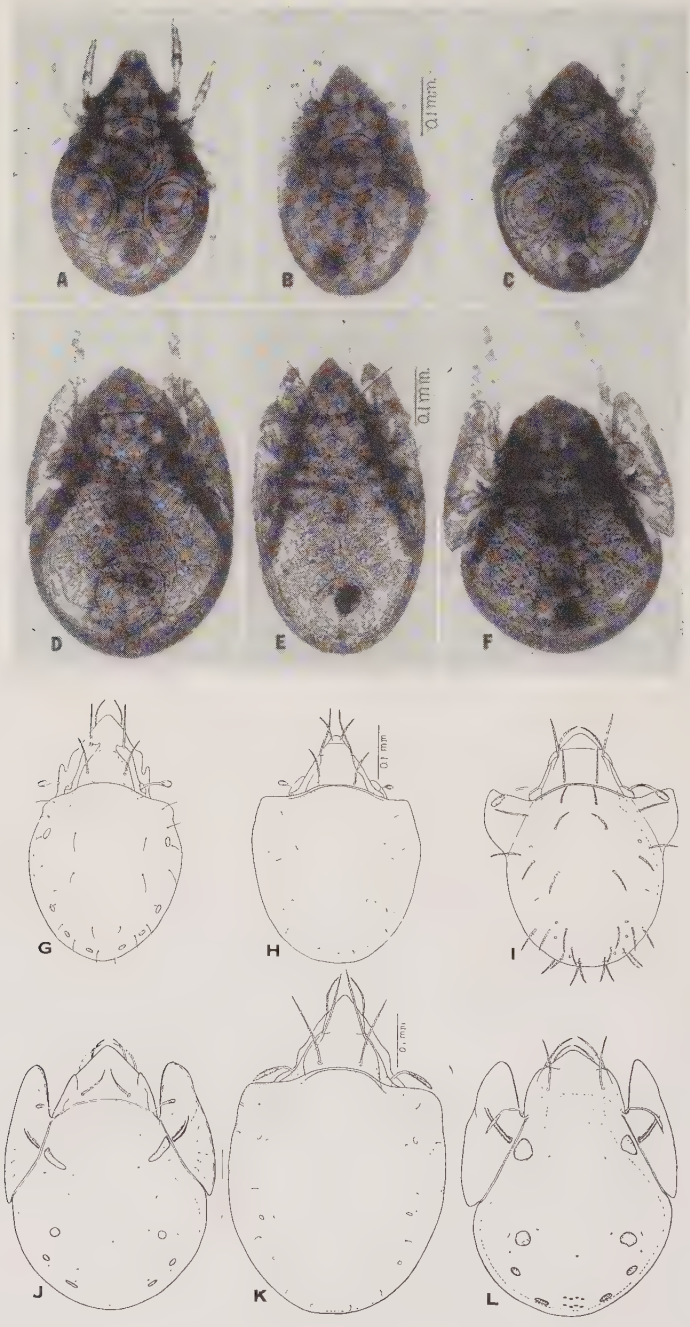


Fig. 1. A & G—*Oribatula minuta* (Ewing, 1909); B & H—*Protoschelobates seghettii* Runkel & Kates, 1947; C & I—*Peloribates curtipilus* Jacot, 1937; D & J—*Galumna emarginatum* (Banks, 1895); E & K—*Scheloribates laevigatus* (Koch, 1836); F & L—*Galumna virginiensis* Jacot, 1929. A to F are photographs (dorsal view) of mounted unstained mites. A, B and C are adult mites containing cysticercoids. D, E, and F are photographs of young mites, fully developed except for additional thickening and pigmentation of the exoskeleton; adult mites of these 3 species are difficult to clear sufficiently for photographing. G to L—sketches of same mites shown in photographs, dorsal view, legs omitted.

before that for only brief intervals, was well shaded by three pine trees, and bounded on one side by woodland. It, therefore, represented to some extent an environment transitional between a woodland or forest habitat and an open sheep pasture. It was not surprising that the oribatid mites collected therefrom presented a different faunal picture than collections from open permanent sheep pastures. The most numerous species in our collections was *G. curvum,* but no quantitative records were kept for this species as it was consistently free of cysticercoids. The numbers of the five species of *Moniezia* vectors varied greatly for the different turf samples. Detailed collection records were kept for only the two galumnid vectors, *G. virginiensis* and *G. emarginatum.* Collections were made of the three small, non-galumnid vectors only for the purpose of determining the incidence of infection with cysticercoids.

The combined average number of *G. virginiensis* and *G. emarginatum* per square foot of turf was not high, amounting to slightly more than 19. It is likely that the numbers of these mites may have been considerably reduced as a result of deaths caused by heavy infections of tapeworm larvae. These small mites are definitely limited in their capacity for harboring cysticercoids, being only about 0.5

TABLE 2.—*Distribution of 2 species of galumnid mites in turf of a pasture plot, 20 by 60 feet, at Beltsville, Md. Plot divided into 3 equal sections longitudinally; section 1 adjacent to pasture, section 2 central section, section 3 adjacent to woodland. Mite collections made during spring and summer of 1947;* (G. v.)—Galumna virginiensis (G. e.)—Galumna emarginatum.

Square feet of turf	Section 1		Section 2		Section 3		Totals	
	G. v.	G. e.	G. v.	G. e.	G. v.	G. e.	G. v.	G. e.
311	3,135	221	507	667	474	1,076	4,116	1,964
Ratio of G. v to G. e.	14 to 1		1 to 1.3		1 to 2.2		2 to 1	

mm. in length, but not in the number of oncospheres they may ingest. The distribution of these two species on the plot proved interesting (Table 2). Early in our collections it was noted that a larger number of *G. virginiensis* were recovered from turf cut farthest from the fence separating the pasture from the adjacent woodland (Table 2, sec. 1), and more *G. emarginatum* were obtained from the part of the plot bordering the woodland (sec. 3). In the short distance of 20 feet the ratio of *G. virginiensis* to *G. emarginatum* changed from 14: 1 to 1: 2.2, the quantities of the two species being about equal in the central section. These data added to those obtained from the Beltsville permanent pasture indicate that *G. virginiensis* is primarily adapted to open permanent sheep pastures at the Agricultural Research Center, Beltsville, Md., while *G. emarginatum* prefers a more protected forest type of environment.

The three non-galumnid vectors were most numerous in collections from section 3 bordering the woodland; *O. minuta* was the most numerous species, *P. seghettii* next, and *P. curtipilus* the least abundant. In the Beltsville area these mites appear to be primarily forest species, as they do not commonly occur in quantities on open permanent sheep pasture, preferring a shaded, protected habitat.

CYSTICERCOID INFECTION RATES AND CAPACITIES OF MITE VECTORS

Cysticercoid infection rates.—With the exception of the difference in mite species, the data on mite infection with *Moniezia* cysticercoids present a similar picture for the two open permanent sheep pastures (Table 3, A), as 3.9 per cent of 34,224 *G. virginiensis* examined from pasture A at Beltsville, Md., and 2.8 per cent

of 1,456 *S. laevigatus* from pastures B at Newell, S. Dak., were infected with cysticercoids. Furthermore, on the basis of estimated numbers of these two species per acre and the average number of cysticercoids recovered per 100 mites collected, it was calculated that both pastures carried a minimum of over 400,000 cysticercoids per acre, a more than adequate number to produce heavy infections in ruminants grazed thereon. These data show to what extent tapeworm cysticercoid infections in mites occur on small permanent pastures grazed more or less continually by sheep for a period of several years.

Markedly different infection rates were obtained for the five species of mite vectors recovered from pasture plot C at Beltsville, Md. (Table 3, B). Cysticercoid infection rates for five different vectors from this plot were: *G. virginiensis* 34 per cent, *G. emarginatum* 11 per cent, *O. minuta, P. curtipilus* and *P. seghettii* (data combined) 6 per cent. These infection rates indicate that of these five mite species *G. virginiensis* is the most efficient vector of *M. expansa,* having

TABLE 3.—*Infection rates on 6 species of Oribatid mite vectors of* M. expansa

Mite species	Mites examined	Mites infected	Per cent of mites infected	Cysticercoids recovered	Cysticercoids per 100 mites
A. Mites collected from permanent sheep pastures					
Galumna virginiensis (ARC, Beltsville, Md.)	34,224	1,338	3.9	2,439	7
Scheloribates laevigatus (Newell, S. Dak.) by Dr. R. T. Habermann	1,456	42	2.8	64	4
B. Mites collected from inoculated pasture plot, Beltsville, Md.					
Galumna virginiensis	4,017	1,395	34	3,873	96
Galumna emarginatum	1,892	217	11	378	20
Oribatula minuta	866	55	6	87	10
Peloribates curtipilus					
Protoschelobates seghettii					
(Data combined)					

three times the infection rate of *G. emarginatum* and nearly six times the combined rates of the three small non-galumnid species. Thus, by collecting only *G. virginiensis* from this plot we were able to secure relatively large quantities of cysticercoids with a minimum of effort.

Cysticercoid capacities of mites.—Not only were variations in infection rates of the different species of mites noted, but also in their capacities of harboring cysticercoids. Generally, the maximum cysticercoid capacity of a mite species appeared to be in direct proportion to its size. For example, the maximum number of cysticercoids recovered from one specimen of the different species follows: The three relatively large species, *G. virginiensis* 13, *G. emarginatum* 12 and *S. laevigatus* 5; the three smaller species, *O. minuta, P. curtipilus* and *P. seghettii* 4 (Fig. 2). *S. laevigatus* is approximately the same size as the two galumnid species (Fig. 1) and probably has a similar capacity for cysticercoids, but the number dissected may not have been large enough to show the maximum cysticercoid infection. From Fig. 1, A, B, and C, showing cysticercoids in the three smaller species, it is obvious that four cysticercoids represent near maximum capacity of these mites.

If fully developed cysticercoids were approximately the same size, it would not be difficult to estimate the maximum cysticercoid capacity of a particular mite species. Generally, however, the greater the number of cysticercoids in a mite the smaller they are. For example, the average length and width of 17 cysticercoids obtained from mites infected with one or two cysticercoids each were 180 by 159 microns, while measurements of six cysticercoids from one mite averaged only 133 by 114 microns and 11 cysticercoids from one mite averaged only 131 by 106 microns. The largest cysticercoid observed was one from a specimen of *G. emarginatum;* the cyst containing the scolex was spherical and measured 218 microns in diameter, while the total length of the larva including its attached cercomere was 737 microns, half again as long as the mite. It was also noted in our dissections that gravid mites seldom contained many cysticercoids, as they usually were filled with eggs. Although not observed, it is possible that development of numerous tapeworm larvae and mite eggs simultaneously, or a hyperin-

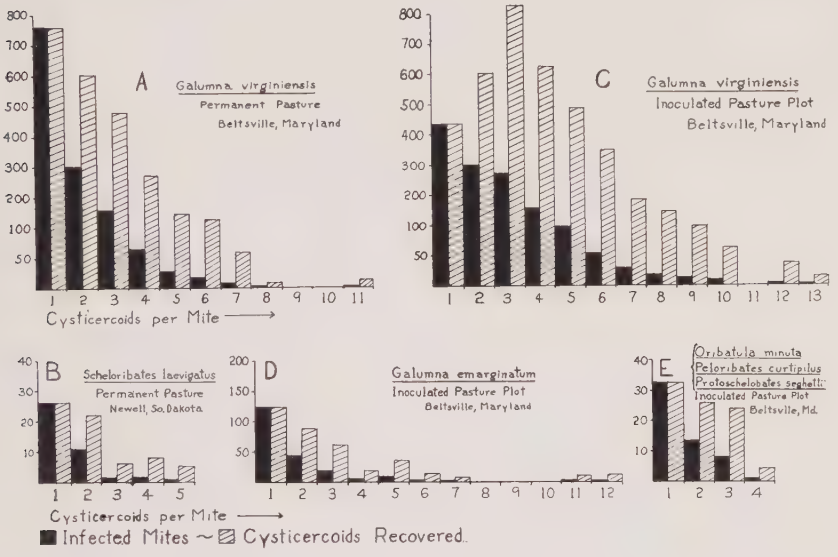


FIG. 2. Breakdown of data on *infected mites only* collected from the indicated sources. Relative cysticercoid infections and cysticercoid capacities are shown.

fection of tapeworm larvae alone, may be fatal to the mites, as their body cavities have limited capacities.

Stunkard (1943) stated "ingestion of oncospheres is accidental and incidental in the feeding habits of the intermediate hosts"; our observations support this view (Fig. 1). Although generally the overall incidence of infected mites was low, the number infected with two cysticercoids was approximately $\frac{1}{2}$ the number with one, the number with three about $\frac{1}{2}$ the number with two, etc., thus indicating that ingestion of oncospheres by mites is largely a matter of chance during their feeding upon dung or organic matter in the soil. This relationship held in all cases except that of *G. virginiensis* collected from pasture plot C (Fig. 2, C). In this case a larger proportion of mites had multiple cysticercoid infections than in the other cases illustrated in Fig. 2. A partial explanation of this exception may be that this species is more coprophagous than the others and thus acquired a greater proportion of multiple infections under the condition of high tapeworm egg concentration on this plot.

DISCUSSION

Oribatid Mites and Transmission of Anoplocephaline Cestodes

To date there is no satisfactory summary available of published information on the rôle of oribatid mites, as a group, in transmission of anoplocephaline cestodes. The general status of our knowledge of speciation of anoplocephaline cestodes is good, but the same cannot be stated of their mite intermediate hosts.

In Table 4 an attempt is made to summarize briefly what is now known concerning the rôle of oribatid mites in the transmission of these parasites. To date approximately 18 genera and 25 species of oribatid mites, belonging to 8 families, have been reported by American and Russian workers as certain or possible vectors of 9 species of anoplocephaline cestodes. The notation below Table 4 shows that complete larval development was not observed in all species listed and, furthermore, the life cycles of the cestodes were determined by feeding cysticercoids to definitive hosts in only a few cases.

It is possible to conclude from this tabulation that there is little intermediate host specificity in regard to the various anoplocephaline cestode species. It is likely, therefore, that by further search many more oribatid vectors will be found. Demonstration in the laboratory of larval development of tapeworms in certain species of mites does not necessarily mean these species are the ones concerned in natural transmission, as natural vectors must be adapted to living in an environment where the definitive host normally obtains its food. Evidence is presented in this paper which shows that the number of mite species concerned in transmission of *M. expansa* on a particular permanent sheep pasture is limited, as only one species from each of two permanent sheep pastures was found infected with cysticercoids.

Although more information is now available on mite vectors of *M. expansa* than on those of other anoplocephaline cestodes, the information is still decidedly fragmentary from a geographical point of view. So few studies have been made on mites concerned in natural transmission that it is impossible to predict what species are present on pastures widely separated geographically or even from pasture to pasture in the same locality. Certain vectors may have a wide distribution, as *S. laevigatus,* which is known to occur in Russia, Germany and the U. S. A. In view of the complexity of the oribatid mite fauna, the large number of known and unidentified species and the present status of our knowledge of mite vectors of anoplocephaline cestodes, it is evident that the mite–cestode relationship provides a fertile field for further study. The summary provided in Table 4 represents only an introduction to the general problem.

Comments on Mite Vectors of M. expansa

Although larval stages of *M. expansa* have been found to develop in several species of galumnid mites by various authors (Table 4), only two naturally infected species collected from pastures have been reported, namely, *G. emarginatum* reported by Krull (1939) and *G. virginiensis* reported in the present paper. We have shown, furthermore, that only the latter species is the principal vector on an open sheep pasture at Beltsville, Md. We still know very little of the rôle played by different species of galumnid mites in various parts of this country, or the world over, in the transmission of *M. expansa.* We do know galumnid mites are widely distributed, but we lack knowledge of their distribution in specific grazing areas. We have found that the above two galumnid species, although closely related morphologically, differ in their distribution on a given pasture and in their reaction to tactile stimuli. *G. emarginatum* was not found on an open permanent sheep pasture at Beltsville, Md., but *G. virginiensis* was present. The former

TABLE 4.—*List of Oribatid mites, reported as vectors of Anoplocephaline cestodes*

Family, genus & species	Author of report	Region	Anoplocephaline cestodes
GALUMNIDAE			
Galumna sp.	Stunkard, 1937	U.S.A.	*Moniezia expansa*—2
do do	Stoll, 1938	do	do —2
do do	Stunkard, 1940	Germany	*Bertiella studeri*—2
do *nigra*	Stoll, 1938	U.S.A.	*M. expansa*—2
do *emarginatum*	Krull, 1939	do	do —3
do *obvius*	Potemkina, 1941	U.S.S.R.	do —2
do do	do , 1944	do	*M. benedeni*—2
do do	Stunkard, 1941	Germany	*Cittotaenia ctenoides*—2
do do	Bashkirova, 1941	U.S.S.R.	*Anoplocephala perfoliata*—2
do do	do	do	*Paranoplocephala mamillana*—2
do *nervosus*	Stunkard, 1941	Germany	*C. ctenoides*—1
do do	Bashkirova, 1941	U.S.S.R.	*A. perfoliata*—2
do *virginiensis*	Kates & Runkel, 1947	U.S.A.	*M. expansa*—3
Allogalumna longipluma	Bashkirova, 1941	U.S.S.R.	*P. mamillana*—2
SCHELORIBATIDAE (Ceratozetidae *s. str.*)			
Scheloribates laevigatus	Stunkard, 1940	Germany	*B. studeri*—2
do do	do , 1941	do	*C. ctenoides*—3; *C. denticulata*—1
do do	Potemkina, 1941	U.S.S.R.	*M. expansa*—2
do do	Kates & Runkel, 1947	U.S.A.	do —3
do do	Potemkina, 1944	U.S.S.R.	*M. benedeni*—2
do do	Bashkirova, 1941	do	*A. perfoliata; A. magna*—2
do *latipis*	Potemkina, 1944	do	*Thysaniezia giardi*—2
do do	Bashkirova, 1941	do	*A. perfoliata; A. magna*—2
Protoschelobates seghettii	Potemkina, 1944	do	*T. giardi*—2
	Runkel & Kates, 1947	U.S.A.	*M. expansa*—3
CARABODIDAE			
Scutovertex minutus	Stunkard, 1940	Germany	*B. studeri*— 1
do do	do , 1941	do	*C. ctenoides*—2; *C. denticulata*—3
Xenillus tegeocranus	do	do	do —2; do —1
Cepheus cepheiformis	do	do	do —1; do —1
Carabodidae sp.	Bashkirova, 1941	U.S.S.R.	*A. perfoliata*—2

TABLE 4. *Continued*

Family, genus & species	Author of report	Region	Anoplocephaline cestodes
NOTASPIDIDAE			
Notaspis coleoptratus	Stunkard, 1940	Germany	*B. studeri*—1
do do	do , 1941	do	*C. ctenoides*—2
Trichoribates incisellus	do	do	do —1
Achipteria (*Notaspis*) sp.	Bashkirova, 1941	U.S.S.R.	*A. perfoliata*—1
PELOPSIDAE			
Pelops tardus	Stunkard, 1941	Germany	*C. ctenoides*—3
do *acromius*	do	do	do —2
ORIBATULIDAE			
Liebstadia similis	do	do	do —1
Oribatula minuta	Kates & Runkel, 1947	U.S.A.	*M. expansa*—3
LIACARIDAE			
Liacarus coracinus	Stunkard, 1941	Germany	*C. ctenoides; C. denticulata*—1
Liacaridae sp.	Bashkirova, 1941	U.S.S.R.	*A. perfoliata*—1
Adoristes ovatus	Potemkina, 1946	do	*Moniezia* sp. (No data)
HAPLOZETIDAE			
Peloribates curtipilus	Kates & Runkel, 1947	U.S.A.	*M. expansa*—3

Explanation of numbers following names of anoplocephaline cestodes: 1. Tapeworm larval development in mites fed eggs in laboratory, but no cysticercoids found. 2. Cysticercoids found in mites fed eggs in laboratory. 3. Cysticercoids recovered from naturally infected mites.

species makes a protective response to tactile stimuli by rapidly snapping shut its pteromorphae over its legs. *G. virginiensis* does not react in this manner. These differences, in addition to the distribution data presented in Table 2 and infection data presented in Table 3, B, indicate that *G. virginiensis* is better adapted to the transmission of *M. expansa* on pastures than is *G. emarginatum*. Thus, different species of vectors of *M. expansa* and possibly other anoplocephalines, are not necessarily equally efficient as intermediate hosts.

Another interesting species is *S. laevigatus*, found by various authors (Table 4) to act as a vector of *M. expansa* and certain other related cestodes in Germany and Russia, and reported by us from South Dakota. Jacot (personal communication from Dr. E. W. Baker) also collected this species from New England. Evidently this species and others belonging to the same family [*P. seghettii* reported by us from Maryland and collected by Dr. Lee Seghetti from a sheep pasture in eastern Montana (personal communication)] also have a wide distribution. To the question as to what factors are concerned in the distribution of various mite vectors of *M. expansa* we have only an incomplete answer. Some important factors may be differences in climate, type of pasture or range, quality and quantity of forage, use and age of pasture, etc. When a pasture is first established from non-pasture land, a certain mite fauna is already present in the humus and soil. Changes which take place during the pasture formation and later in its use by sheep probably profoundly alter the fauna. This may result from alterations in the organic content of the soil upon which the mites feed. Mite species able to survive under the new conditions may increase greatly in numbers and eventually one or more species become the principal vectors of tapeworms on that pasture. Theoretically, therefore, it may be assumed that vectors present in great numbers on a permanent pasture were present in the soil before the pasture was formed. The mite fauna in pasture turf is probably not static, but may alter from time to time depending on the general condition and use to which the pasture is put.

SUMMARY

1. Six species of oribatid mites, belonging to five genera and four families, have been found to be vectors of the sheep tapeworm, *Moniezia expansa*. Four vectors, namely, *Galumna virginiensis*, *Oribatula minuta*, *Peloribates curtipilus*, and *Protoschelobates seghettii*, have not been reported as vectors of this tapeworm prior to our collections. One species, *Scheloribates laevigatus*, is reported as a vector for the first time from the United States. These mites are illustrated by drawings and photographs, *G. emarginatum*, a previously reported vector, being included for comparison.

2. Observations are reported on the systematic position, distribution and ecology, cysticercoid infection rates, and cysticercoid capacities of these mites.

3. *G. virginiensis* was the only vector collected from an open permanent sheep pasture at Beltsville, Md.; 133 mites were collected per square foot of turf with an average cysticercoid infection rate of 3.9 per cent. It is estimated that an acre of this pasture contained a minimum of 6,000,000 mites of this species infected with over 400,000 cysticercoids. The maximum number of cysticercoids recovered from one mite was 13.

4. *S. laevigatus* was the only vector collected from an open permanent sheep pasture at Newell, S. Dak.; 221 mites were recovered per square foot of turf with an average cysticercoid infection rate of 2.8 per cent. It is estimated that a minimum population of over 9,000,000 mites infected with over 400,000 cysticercoids was present on this pasture. The maximum number of cysticercoids recovered from one mite was 5.

5. On a small pasture plot droppings containing *M. expansa* eggs were placed and five of the six mite species (*S. laevigatus* excluded) reported as vectors in the present paper were found infected with cysticercoids the following year. Cysticercoid infection rates were as follows: *G. virginiensis* 34 per cent, *G. emarginatum* 11 per cent, *O. minuta*, *P. curtipilus* and *P. seghettii* (data combined) 6 per cent. The maximum number of cysticercoids recovered from one specimen of *G. emarginatum* was 12; from the latter three small species the maximum number was 4.

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Tortugaster fistulatus, n. gen., n. sp., a Rhizocephalan Parasite of *Munidopsis robusta*

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Rhizocephalids of the family Peltogasteridae are known as parasites of the Paguridae or hermit crabs. The chief exception thus far noted is the genus *Galatheascus* which Boschma (1929) described from specimens found infecting *Galathea*, an anomuran crab of the family Galatheidae. This was surprising, since the galatheids are generally parasitized by representatives of the Lerñaeodiscidae.

The genus *Galatheascus* is characterized primarily by having the long axis of the parasite perpendicular to the main axis of the host. Details of internal anatomy are very similar to those of *Peltogaster* and the larvae hatch in the nauplius stage. Two species of *Galatheascus* are known, viz., *G. striatus* (Boschma, 1929) and *G. minutus* (Boschma, 1933).

From the collections of the U. S. National Museum, the writer recently received on loan for study two specimens of *Munidopsis robusta* A. Milne-Edwards (family Galatheidae), each with a rhizocephalid attached to the abdomen. These specimens had been collected by Dr. Waldo L. Schmitt at Tortugas, Florida. Both parasites are definitely representatives of the Peltogasteridae, but although they lie with the long axis perpendicular to that of the host, they cannot be placed in the genus *Galatheascus* from which they differ in important anatomical respects and in the

fact that the larvae hatch in the cypris stage. It is therefore necessary to establish a new genus which we propose to call *Tortugaster*.

Tortugaster, n. gen.

Diagnosis.—Solitary, body elongate and oriented with long axis perpendicular to that of host. Mantle opening at the anterior end; stalk in the median region. Colleteric glands simple; testes tubular; both situated in vicinity of stalk. Vasa deferentia convoluted, wider than testes, with internal ridges, opening posteriorly. Larvae hatch in the cypris stage. On Galatheidae.

Genotype.—Tortugaster fistulatus, n. sp.

This animal is apparently the only representative of the family Peltogasteridae having vasa deferentia with internal ridges, a character found commonly in the Sacculinidae. It is likewise the first peltogasterid known to hatch in the cypris stage. This feature has hitherto been found only in *Clistosaccus* (family Clistosaccidae), *Sylon* (family Sylonidae), *Sesarmaxenos* and *Ptychascus* (family Sacculinidae), and *Thompsonia* (family Uncertain).

Tortugaster fistulatus, n. sp.

Cotypes.—Off Tortugas, Florida, 220 fathoms, July 31, 1930; one specimen on *Munidopsis robusta* A. Milne-Edwards, W. L. Schmitt coll. Off Tortugas, Florida, 280 fathoms, August 5, 1932; one specimen on *Munidopsis robusta* A. Milne-Edwards, W. L. Schmitt coll. U.S.N.M. 119885.

Transverse sections were made of both specimens, and one set of sections has been deposited in the collections of the U. S. National Museum.

Diagnosis.—Internal cuticle with retinacula consisting of one or two smooth spindles having a length of 6 to 9 μ. Stalk removed far to the left of the mid-dorsal line, with the male genital organs to the right of the stalk. Visceral mass abbreviated, absent in posterior third of animal (hence the name *fistulatus*, meaning "hollow").

Description.—The parasites were attached to the terminal segment of the crab's abdomen, on the ventral side. They were oriented with mantle opening directed toward the right side of the host and with their long axis perpendicular to that of the host (Fig. 1, A).

One specimen (Fig. 1, C), occurring on the *Munidopsis* which was collected July 31, 1930, had a length of 9 mm., a width, taken in the region of the stalk, of 5 mm., and a thickness (dorso-ventral diameter) of 3.5 to 4 mm. The other specimen (Fig. 1, A) measured 10 mm. in length, 6 mm. in width, and 4.5 mm. in thickness. In shape, both parasites, particularly the smaller specimen, resembled a mature *Peltogaster*, with the right side convex and the left side concave. The larger specimen had the posterior third reflexed against the concave side. This posterior lobe is a region of the animal extending beyond the limits of the visceral mass and hence is softer and more susceptible to folding.

The mantle opening, which occurs at the anterior end, is relatively small, but is surrounded by a thick, elevated cushion formed by the sphincter. The stalk of attachment is approximately equidistant from anterior and posterior ends but is peculiar in being shifted considerably to the left of the mid-dorsal line.

The mantle is well developed, and in general like that of *Peltogaster paguri* Rathke except that the muscle tissue is less pronounced. The external cuticle is thin, measuring 5 to 8 μ in thickness. It bears no excrescences, but in transverse sections shows numerous small indentations. In the region of the stalk the external cuticle increases in thickness, reaching a maximum of 30 μ in the wall of the stalk itself. It does not, however, form a definite shield around the base of the stalk as

is the case in *P. paguri,* although some thickening of this area is visible in the sections.

The internal cuticle is thin and bears retinacula (Fig. 1, D) consisting of one or two blunt spindles arising from a slightly elevated base. The height of the spindles varies from 6 to 9 μ and the width from 2.3 μ to 3 μ.

The visceral mass is broadly attached to the mantle only in the region of the male genital organs. Its attachment begins at the sphincter, into which the visceral mass projects. From this point the mesenterial attachment gradually widens as it approaches the stalk. It remains wide in the testicular region, narrows slightly in the region of the vasa deferentia, then terminates rather abruptly just posterior

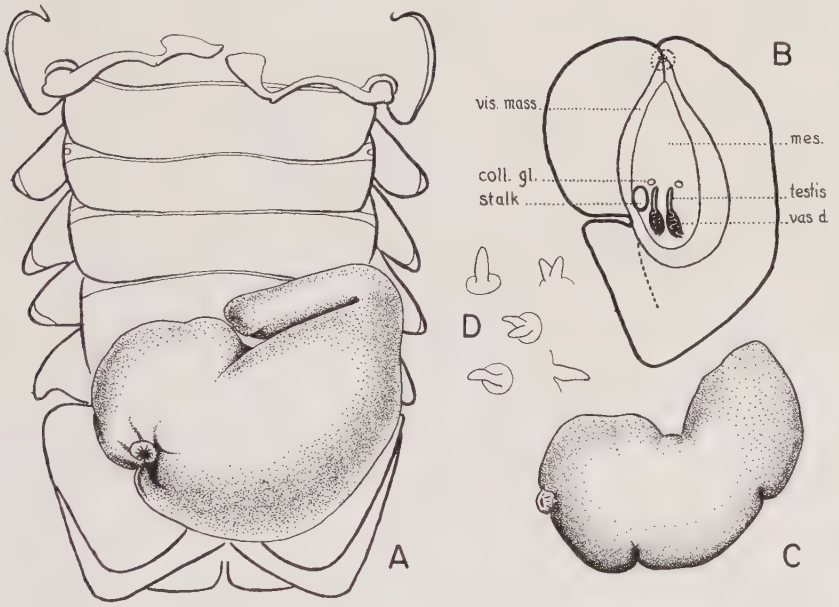

FIG. 1. *Tortugaster fistulatus,* n. gen., n. sp. A—Parasite attached to abdomen of *Munidopsis robusta* A. Milne-Edwards from Tortugas, Florida. B—Diagram of same specimen, viewed from the dorsal surface, to show position of stalk and internal organs. C—Another specimen, 9 mm. in length, from same host and same locality, shown in ventral view. D—Types of retinacula present on internal cuticle. *Coll. gl.,* colleteric gland; *mes.,* mesentery; *vas d.,* vas deferens; *vis. mass,* visceral mass.

to the openings of the vasa deferentia. In the larger specimen, the posterior third of the animal, which is strongly reflexed against the left side, does not contain any part of the visceral mass.

In transverse sections the visceral mass is seen to be placed obliquely in the mantle cavity (Fig. 2). This is due to the fact that the mesenterial attachment is shifted to the left of the median antero-posterior axis. The bulk of the visceral mass inclines toward the right side of the animal. The stalk, instead of being in the center of the mesenterial zone, is at its extreme left.

In the smaller specimen, which has eggs in the mantle cavity, the visceral mass is empty, except for a few branching egg cords containing small oogonia. In the larger specimen, which has cypris larvae in the mantle cavity, the visceral mass is solidly packed with ripe eggs. The musculature in both cases is very scanty.

The ganglion, difficult to find, is a small quadrangular mass lying in the dorsal

portion of the visceral mass in front of the stalk. It immediately precedes the colleteric glands.

The colleteric glands are simple tubes with an undivided lumen, emptying into the angle of the mantle cavity formed by the junction of the visceral mass with the mantle. They occupy a position immediately in front of the testes and may overlap the anterior ends of these organs. Both are on a level with the anterior edge of the stalk. In the specimen with empty visceral mass, the colleteric glands are thin-walled spaces that appear to be mere vestibula of the egg tubes. They are irregularly elliptical in cross-section. In the other specimen, because the visceral mass is crowded with large eggs, the colleteric glands are collapsed. The one on the left is almost indistinguishable, while the gland on the right is so compressed that the lumen is practically obliterated and the wall appears to be of ten or more cells in thickness. The apparent thickness of the wall is obviously the result of the highly collapsed condition of the gland.

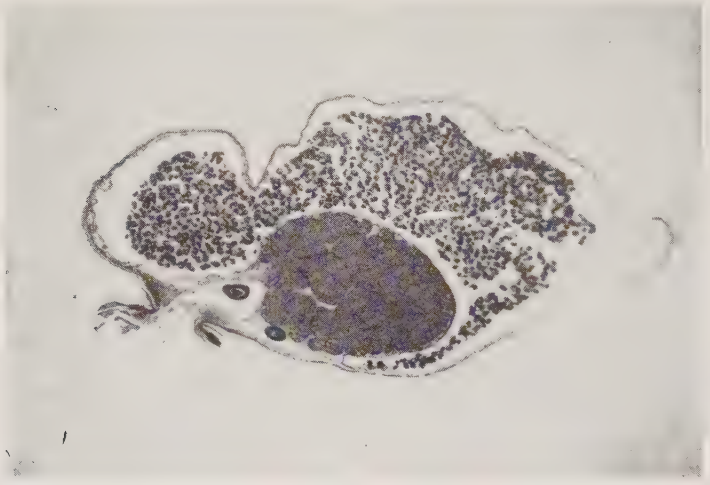

FIG. 2. *Tortugaster fistulatus*, n. gen., n. sp. Photomicrograph of transverse section in the region of the testes to show tilting of visceral mass to the right and displacement of stalk to the extreme left of the mesentery in which the testes are located. The mantle cavity is filled with cypris larvae. Section made from specimen shown in Fig. 1, A. ×10.

The testes are slightly more dorsal in position than the colleteric glands and lie in the mesenterial region of the visceral mass. The left testis begins a little in advance of the one on the right. They are straight or slightly bent tubes, whose length, in the case of the left testis, reaches from the anterior to the posterior margin of the stalk. The right testis, because it begins farther backwards, extends a little beyond the posterior edge of the stalk. Both testes lie to the right of the stalk (Fig. 2) and are separated from each other by a fairly wide intervening space.

Each testis begins as a solid cord, but soon exhibits a large central lumen. In general, the wall of the testis is made up of a thin outer connective tissue layer, a thick epithelial layer with cells arranged circularly with respect to the lumen, and an inner layer of hypertrophied cells with degenerating nuclei forming the so-called "honeycomb." There is no basement membrane present. The outer and middle layers occur in all four testes, but the honeycomb layer is poorly developed in one specimen, and its presence seems to depend on the physiological

condition of the testis. It is best developed in the left testis of the specimen which has cypris in the mantle cavity. This testis is devoid of sperm, whereas the right testis of the same animal is filled with masses of ripe spermatozoa, and here the hypertrophied layer is scanty.

Where the testis merges into the vas deferens the wall becomes thicker and the lumen diminishes in size. This short transition zone is followed by the vas deferens proper where the wall is again thinner and now made up of tall columnar cells standing radially with reference to the lumen. In contrast to the testis, the vas deferens has a much expanded lumen with strong ridges or folds projecting into it (Fig. 3). The vas, moreover, is convoluted, with the coils pressed together so that no intervening connective tissue spaces appear between them.

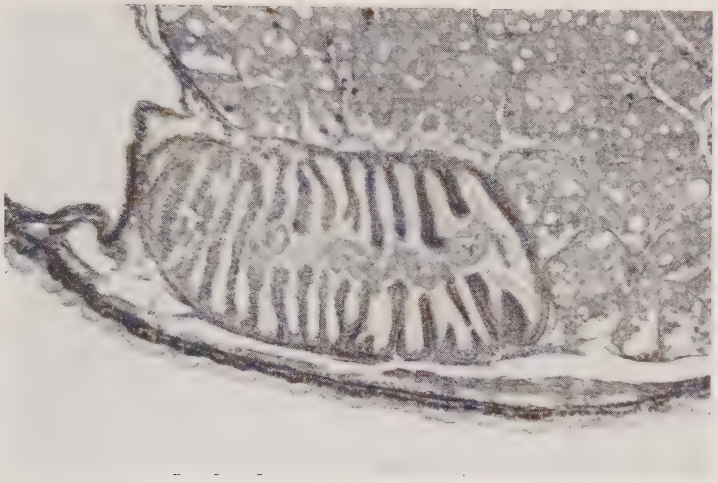

FIG. 3. Photomicrograph from same series as Fig. 2 showing the vas deferens near its termination. Note the strong folds projecting into the lumen, which, at this point, is plugged with chitin. ×84.

The extent of the vas deferens, in its natural unravelled condition, is about one-third greater than the length of the testis. Its great development, with high internal ridges projecting into a wide lumen, a characteristic not commonly found in the Peltogasteridae, causes the vas over part of its length to protrude beyond the lateral surface of the visceral mass. It terminates on the summit of a prominent papilla which projects into the mantle cavity.

The contents of the mantle cavity, as stated above, differ in the two specimens available for examination. In one the cavity is filled with eggs that were undergoing fertilization at the time the animal was killed. These eggs are comparatively large and oblong, measuring 150 to 175 μ in length and 70 to 75 μ in width. Fortunately, the other specimen contains embryos near the termination of their development. They are well-formed cypris larvae, having a length of about 200 μ, a width of about 90 μ and a thickness of about 100 μ.

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Observations on the Viability of Eggs of *Ascaris* Removed from Swine by Treatment with Sodium Fluoride

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Although the major objective in anthelmintic treatment is accomplished by removing the parasite from the host, a factor of no small importance is the fate of the many fertile eggs in the uteri of female worms that are removed. This is especially true with *Ascaris,* which, because of its large size, may harbor many thousands of eggs, and because these eggs normally remain viable, under natural conditions, for long periods of time.

In view of the frequency with which sodium fluoride is now being used for the removal of ascarids from swine, it was considered desirable to ascertain the effect of this compound on the eggs of the expelled worms. This paper summarizes some experiments that were carried out at Tifton, Georgia and Chicago, Illinois, in order to obtain some information on this point. Thanks are due Mr. B. L. Southwell of the Georgia Coastal Plain Experiment Station, Tifton, Georgia, and Dr. H. E. Kingman, Jr., of Chicago, Illinois, for their part in making this work possible.

Pigs weighing from 186 to 206 pounds were fed for one day a ration containing 1 per cent by weight of sodium fluoride. For several days following treatment all ascarids passed were collected, and cultures were made of the eggs from the uteri of the female worms in 1 or 2 per cent formalin, or tap water. Eggs were also cultured from the feces of infected swine. All cultures were held for a sufficiently long period of time to permit a study of the development of the eggs. Infectivity was ascertained by feeding some of the embryonated eggs to guinea pigs.

Eight pigs were treated in the experiments at Tifton, and mature female worms were recovered for culturing on the 2nd, 3rd, 4th, and 8th days. Observations of the eggs every second day for 13 days after the last culture was made showed no retardation of development in the great majority of the embryos.

In the Chicago experiments, mature female worms were recovered for culturing from 2 pigs on the first 5 days following treatment. Observations between the 25th and 30th days after the cultures were made showed that a high percentage of eggs in all cultures were embryonated. Furthermore, embryos from 2 cultures made from worms passed 4 and 5 days after treatment produced heavy infections (approximately 780 and 2,900 larvae, respectively) in the lungs of 2 guinea pigs to which they were fed.

The animals used in the Chicago tests, including 1 control pig, were autopsied after a post-treatment holding period of 6 days. All 24 ascarids were removed from 1 pig and 77 per cent, or 20 of 26 worms, from the other. None of 8 ascarids were eliminated by the control pig.

Of some interest is the fact that eggs in cultures made from worms remaining in the one treated pig did not embryonate, while those made from the control pig developed normally. This finding is probably not significant because the female worms remaining in the treated pig were small. Furthermore, it is not supported by other data obtained in this experiment. It is very doubtful, therefore, that the sodium fluoride treatment was responsible for the failure of the eggs to develop in this instance.

These results indicate that the eggs in the uteri of ascarids removed from pigs by treatment with one per cent sodium fluoride for one day are not adversely

affected. Such eggs appear to develop in the normal manner and a high percentage of them are viable and infective.

The treatment removed 88 per cent of 50 ascarids from 2 pigs and was well tolerated by the 10 pigs to which it was given.

Another Case of Guinea Worm, *Dracunculus* sp., Infestation in a Dog in the United States

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During November, 1947, the Zoological Division of the Bureau of Animal Industry received from Dr. D. E. Kallman, Henderson, North Carolina, a piece of a worm removed from the hind leg of a dog. In a note accompanying the specimen it was stated that on the leg from which the worm was removed there was an "abscess." The exact location of the "abscess" was not further described nor was it stated whether the worm protruded from the "abscess" and was removed by extraction or whether leg was incised and the worm recovered from the incision. However, further inquiry elicited the information that the dog from which this worm was removed was a two-year-old pointer kept in Durham and Granville Counties, North Carolina, that it appeared to be healthy except for an enlargement of the lower part of one of the hind legs, and that the worm was removed from the "abscess," presumably by extraction.

On examination the specimen was found to consist of a 24-cm long posterior portion of a worm with the tail end resembling in structure and conformation that described and figured for *Dracunculus*. The portion of the worm available for examination contained some larvae. These larvae were compared with those from identified specimens of *Dracunculus* available in the U.S.N.M. Helminthological Collection and on the basis of that comparison they were considered to be larvae of *Dracunculus*.

This worm, therefore, is considered to be a specimen of *Dracunculus* sp., (1) because of its location in the host and the lesions produced, (2) because the tail end, the only portion available for morphological study, resembles that of *Dracunculus medinensis* as figured by Yorke and Maplestone and other authors, and (3) because the larvae found in the portion available for examination, resemble the larvae of identified specimens of *Dracunculus*.

According to Chandler (1942) guinea worms have not been found with any degree of regularity in any mammals, except those mentioned in the paper cited, in the Western Hemisphere. He stated that up to the time of his own report of their occurrence in raccoons in East Texas these worms had been reported 3 times from this host, once each in Pennsylvania (?), New York and Ontario; twice in mink, in Iowa and Nebraska; once in silver fox in Iowa; and once in a house dog in South Dakota.

Goble (1942) reports finding them in the hind legs of a Bonaparte weasel, *Mustela cicognanii cicognanii*, and Erickson (1946) reports their occurrence in a skunk, *Mephitis mephitis*, in Minnesota. The U.S.N.M. Helminthological Collection contains specimens from raccoon in Maryland, mink in New York, silver fox in Wisconsin, muskrats in Maryland and North Dakota, and dog in North Carolina (subject of the present note).

The present host and distribution records are therefore, as follows:

Raccoon.—Ontario (Can.), Maryland, Texas, Pennsylvania (?), New York.

Silver Fox.—Iowa, Wisconsin.

Mink.—Ontario (Can.), Iowa, Nebraska, New York.
Muskrat.—Maryland, North Dakota.
Dog.—South Dakota, North Carolina.
Skunk.—Minnesota.
Weasel.—New York.

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The Dog, *Canis familiaris*, a New Host of *Balantidium* sp.

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U. S. Bureau of Animal Industry

Recently there was received at the Zoological Division, Bureau of Animal Industry, Agricultural Research Center, Beltsville, Maryland, a sample of feces from a dog with a request that it be examined for *Balantidium* sp. This sample was sent to the writer by Dr. A. C. Yow, Henderson, N. C., who stated in his letter of transmittal that the dog from which the sample was obtained was suffering from a severe diarrhea. No specimens of *Balantidium* were found in this sample. In the letter informing Dr. Yow of the result of our examination, it was suggested that if the organisms were present in the sample at the time it was taken, they probably had died and disintegrated during the time it took for the sample to reach Beltsville, Md., since no preservative had been added. It was suggested, furthermore, that in order to detect these organisms in a fecal sample, it would be desirable to examine the sample as soon as possible after it had been voided. In his reply to our letter Dr. Yow informed us that a fecal sample from this dog had been submitted for examination to the State Laboratory of Hygiene, at Raleigh, N. C., and that that laboratory reported having found specimens of *Balantidium coli* in the sample. A copy of the report of that laboratory, giving the result of the examination, was attached to Dr. Yow's letter.

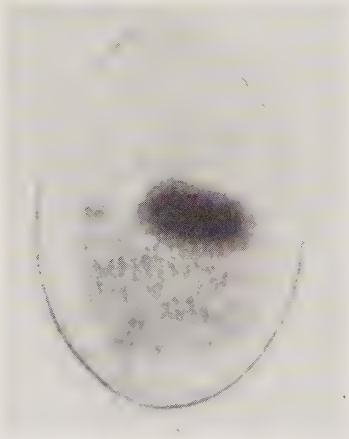

Fig. 1. *Balantidium* sp. from the dog, *Canis familiaris*.

Since, according to the host and parasite records available in the Zoological Division, *Balantidium* has not been previously reported as a parasite of dogs, Dr. Yow was requested to obtain another fecal sample from this dog if possible and, if the stool was liquid as the first specimen had been, to dilute it with about an equal quantity of 5 per cent formalin and forward it to us. We were next informed that the dog had died, but that a sample of the liquid contents of the large intestine had been prepared as suggested and forwarded as requested. Examination of this sample showed a large number of well-preserved and easily recognizable specimens of *Balantidium* sp., (Fig. 1.) In the letter reporting the

death of the dog, Dr. D. E. Kallman, who is associated with Dr. Yow, stated that at necropsy he found ulcerated areas in the intestine. Unfortunately the death of the animal had not been anticipated and, therefore, no tissues showing gross lesions were available to us for histological examination.

The organisms vary from about 0.05 to 0.075 mm. in length and from 0.035 to 0.05 mm. in width.

Hypoderma bovis Larvae from the Esophagus of Sheep

G. DIKMANS

U. S. Bureau of Animal Industry

A piece of tissue containing some fly larvae was recently submitted for examination to the Zoological Division of the Bureau of Animal Industry by Dr. C. L. Davis, Pathological Division, Denver, Colorado. In his letter of transmittal Dr. Davis stated that the piece of tissue was a section of esophagus of an aged ewe slaughtered at Denver, Colorado. This section had been removed at postmortem examination of the carcass and retained for further examination by Dr. H. L. Shorten, because in his previous postmortem inspection of sheep he had not encountered this condition. At Denver the larvae were tentatively identified as those of *Hypoderma lineatum*. At the Zoological Division's station at Beltsville, Maryland, the tissue was examined and some of the larvae were removed and submitted to the Division of Insect Identification, Bureau of Entomology and Plant Quarantine, for determination. Mr. C. T. Greene of that division identified them as larvae of *Hypoderma bovis*, commonly known as the northern heel fly or cattle grub.

Bishopp, Laake and Brundret (1926, U. S. D. A. Bull. 1369: 1–119) describe several attempts to infest goats and sheep with the larvae of *Hypoderma lineatum*. One attempt was made to infest a goat by placing the eggs of the fly on the leg, but these eggs apparently did not hatch. In two other experiments flies were induced to deposit eggs on the legs of two goats. The eggs hatched in both cases but penetration of the larvae was observed in only one. There was no further development of these larvae.

In other experiments larvae of *H. lineatum*, removed from the gullets of cattle, were placed under the skin of the hind legs of three goats. Thirty larvae were placed under the skin of one goat, and 18 and 20 under that of the second and third goats, respectively; the larvae varied from 10 to 15 mm in length. In goat 1, two larvae reached the skin of the back, after 26 days; in goat 2, three larvae reached the skin of the back, two after 13 days and one after 20 days; and in goat 3, two larvae were found in the skin of the back 13 days after insertion under the skin. No goats were found by them to be naturally infested, but they report receiving a series of *Hypoderma* larvae from goats in the Punjab and that among these larvae two were found to be larvae of *Hypoderma lineatum*.

They also attempted to infest sheep in the same manner as they had attempted to infest goats and with about the same results. Eggs deposited on the legs of three sheep failed to develop in two cases but a few eggs hatched and the larvae penetrated in the third case, whereas larvae removed from cattle gullets and inserted under the skin of sheep reached the skin of the back in from 8 to 35 days. In no case, either in the goats or in the sheep, did the larvae reaching the back develop to maturity.

These authors reported that they had never observed a larva of *Hypoderma* on a sheep in nature nor had they seen any indication of the attack of sheep by heel flies.

So far as the writer has been able to determine there are no previous records of the occurrence, under natural conditions, of this parasite in sheep in North America.

The collector, Dr. H. L. Shorten, is to be commended for his alertness in recovering these parasites.

An Outbreak of Hexamitiasis in Turkeys in Virginia

M. M. FARR, E. E. WEHR, and D. S. JAQUETTE
U. S. Bureau of Animal Industry

In August 1946 a farmer living near Clifton, Virginia, brought to the Zoological Division, Agricultural Research Center, Beltsville, Md., several sick turkey poults, about 10 weeks old, for diagnosis. He stated that many of his approximately 200 turkey poults were in poor condition and that several had died within the past several days.

The birds brought to the laboratory were killed and autopsied. The only significant pathological condition found was an intense inflammation of the upper part of the small intestine where large numbers of the flagellate protozoan, *Hexamita meleagridis*, were present. In a subsequent visit to the farm, portions of 3 freshly passed droppings from young turkeys were examined microscopically and found to contain motile *Hexamita*. Moreover, it was observed that the breeder birds were housed at one end of a wooden barn and the poults at the other end, the two areas being connected by a narrow hallway. Apparently no precautions were taken to avoid mechanical transfer of droppings from one end of the barn to the other by those who fed the birds. Presumably the poults acquired the infection from the older birds through ingestion of feed or water contaminated with droppings harboring the parasites.

In order to determine whether *Hexamita* were actually causing the death of turkey poults on the farm, the unused portions of the 3 fecal samples in which the organisms were found, were suspended in physiologic saline and introduced into the crop of each of 9 one-week-old, parasite-free turkey poults brought from Beltsville to the farm for this purpose. The inoculated birds were taken back to Beltsville and placed in clean metal brooders. Four days after inoculation 1 bird died and a few *Hexamita* were found in the upper part of the small intestine, the wall of which was slightly inflamed. Of the remaining 8 birds, 7 died within the next 10 days. At autopsy, 2 showed intense inflammation of the small intestine, which contained *Hexamita* in fairly large numbers; 2 contained *Hexamita* but showed no visible pathological changes; the remaining 3 birds, which died over a week end and were kept in a refrigerator for a time, showed no *Hexamita*, presumably because the organisms had disintegrated during the time elapsing between death and autopsy.

A review of the records on the occurrence of *H. meleagridis* in birds shows that prior to this report the parasite in question had been recorded from California, Connecticut, Illinois, Indiana, Massachusetts, Missouri, New York, North Dakota, Oregon, Utah and Washington.

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